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EFFECT OF SECRETIN AND PILOCARPINE UPON THE BLOOD LIPASE OF DOGS

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The author[1] has shown that the isolated liver is able to produce lipase in response to the perfusion of a weak secretin solution and to markedly alter its lipolytic activity in response to pilocarpine.

The work herein reported shows the influence of secretin and pilocarpine upon the lipolytic activity of the blood in the whole animal.

EXPERIMENTAL METHOD. Adult mongrel dogs, regardless of age or nutritional condition, were used in this work. They were all given their last food about twenty hours previous to the experimental period.

Experimental procedure common to all series. Having sufficiently gentled the dogs used in this research, they submitted without pain or anxiety to the following procedure:

They were held in the dorsal recumbent posture so as to facilitate access to the external jugular vein. After shaving the neck helpful slight pressure was applied to engorge the vein and make it conspicuous. Blood was drawn through a 21 gauge needle. By this procedure 10 cc. samples were collected and substances injected at desired intervals during the experimental period which usually lasted two hours. In two or three instances the vein was exposed to make access to it easier.

At the conclusion of the experiment all of the collected samples were centrifugalized and the lipolytic activity of the serum determined as described later.

Procedure in series 1. This series comprises control experiments to determine the lipase variations in the blood taken at fifteen minute intervals for a period of two hours.

Procedure in series 2. In this series an initial control sample of blood

[1] This Journal, 1923, lxv, 287.

was drawn from the jugular vein, small and large amounts of pilocarpine were immediately injected into the vein and within a minute or two another 10 cc. sample of blood was withdrawn to determine any possible immediate effect of pilocarpine upon the lipase of the blood. Subsequent 10 cc. samples of blood were taken at various intervals throughout the usual two-hour experimental period.

Procedure in series 3. An initial 10 cc. sample of blood was taken, 10 cc. of dilute secretin, slightly alkaline in reaction, were injected and another 10 cc. sample of blood was withdrawn immediately to control the direct effect of secretin upon the blood lipase. At subsequent intervals during the remainder of the period 10 cc. blood samples were taken for determination of changed lipolytic activity.

Preparation and dosage of secretin. The secretin was prepared on the same day of the experiment by boiling in 200 cc. of 0.4 HCl the ground mucosa of the duodenum and upper jejunum of a dog, slightly over neutralizing with KOH and filtering. Ten cubic centimeters of this dilute, filtered solution of secretin were injected intravenously and blood samples subsequently withdrawn.

Blood lipase determination. After completion of the experiment, all of the blood samples taken were centrifugalized at one time. One cubic centimeter samples of the serum were incubated for one hour in ground-glass stoppered weighing tubes with 10 cc. of distilled water, 3 drops of toluol and 2 drops of neutral azo-litmin. The tubes were removed and neutralized with KOH. One-half cubic centimeter of neutral, absolute ethyl butyrate was then added and the tubes incubated at 40°C. At the expiration of twenty-four hours the tubes were removed and their contents titrated against N/50 NaOH to determine the amount of butyric acid liberated by the hydrolysis of the ethyl butyrate.

Checks were run on the amount of ethyl butyrate hydrolyzed by the blood, the amount of acid liberated spontaneously by the blood and the cleavage of ethyl butyrate by water. These factors are all considered and deducted in the preparation of the table of results. The figures given in this paper, therefore, represent the lipolytic activity of 1 cc. of blood serum in terms of N/50 NaOH.

EXPERIMENTAL RESULTS. *Series 1.* During the two hour experimental period the fat-splitting ability of the blood of the normal, unanesthetized dogs did not vary. The variations shown in figure 1 are all within the limits of error of the method.

Series 2. The intravenous administration of 10 cc. of a slightly alkaline solution of secretin produced an appreciable increase in blood lipase in each dog.

The maximum effect was obtained in from fifteen to thirty minutes and ranged from a 25 to a 65 per cent increase (fig. 2); at the expiration of ap-

FIG. 1

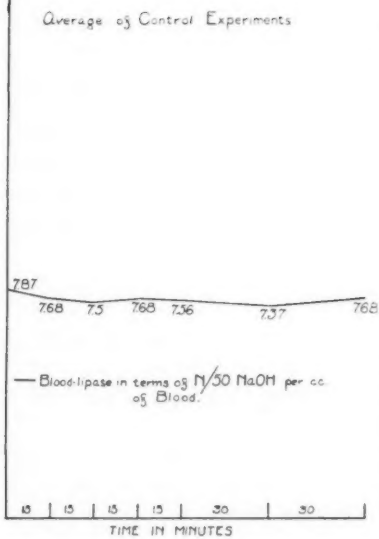


FIG. 2

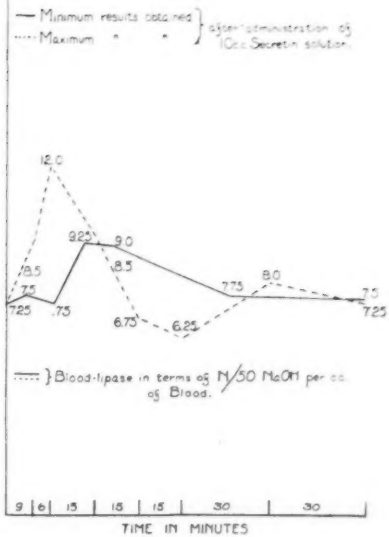


FIG. 3

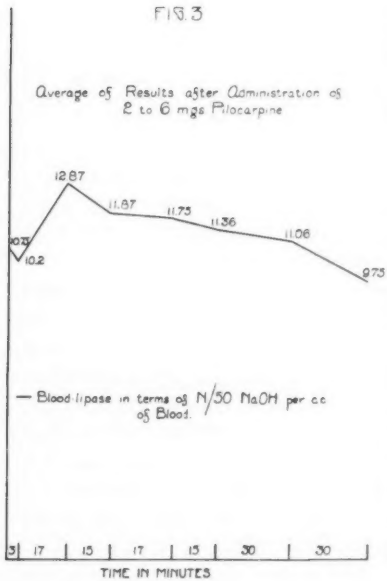
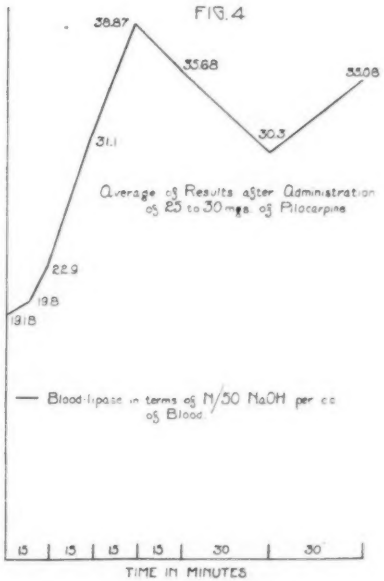


FIG. 4



proximately an hour after injection of the secretin the original level of blood lipase was again reached.

Series 3. Variable results were obtained after the administration of 2 to 6 mgm. of pilocarpine. The dog to which 2 mgm. were given showed no increase in blood lipase. Four milligrams given to another dog gave an increase of approximately 25 per cent. Six milligrams given to still two other dogs gave no increase in one but a 35 per cent increase in the other.

Twenty-five and 30 mgm. doses of pilocarpine injected intravenously gave large increase in the blood lipase in each instance. The increase for the series of four dogs receiving this large dose averaged about 100 per cent.

SUMMARY

1. No variations were noted in the blood lipase of normal unanesthetized dogs.

2. Fresh secretin in 10 cc. doses intravenously increased blood lipase from 25 to 65 per cent in from fifteen to thirty minutes.

3. Pilocarpine increases blood lipase consistently only when the dosage is high. Twenty-five or 30 mgm. of pilocarpine intravenously gave an average increase of approximately 100 per cent.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XX. Reciprocal Size Changes of Gonads and Thyroids in Relation to Season and Ovulation Rate in Pigeons

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Data demonstrating a seasonal variation of thyroid size in male and female pigeons have been recently published by Riddle and Fisher (1925). It is now possible to examine the seasonal changes in gonad size in practically the same group of birds which supplied the data for thyroid size. When the two series of measurements are thus compared it is found that the periods (autumn and winter) of increased thyroid size are periods of decreased gonad size. On the other hand, the seasons (spring and summer) of reduced thyroid size are seasons of gonad enlargement. It is further found that the period of free or frequent ovulation in these same pigeons coincides with the period of diminished thyroid size and of larger gonad size; the period of restricted ovulation—and also of more frequent reduction of the pigeon's clutch to a "single" egg—coincides with the period of increased thyroid and decreased gonad size. It is the purpose of the present paper to record the data which establish these relationships. The question of antagonistic relationships of thyroid and gonad is discussed.

Material and methods. The material which provides the data for gonad and thyroid size has been fully described in the publication on thyroid size cited above. The methods used in collecting the gonad data were the same as for the thyroids. Only gonads and thyroids of birds found at autopsy to be free of disease are considered here. In obtaining data on seasonal rate of ovulation it was obligatory to utilize a selected group of birds. Only those birds which are given opportunity to lay the maximum number of eggs—or which are at least given equal opportunity for frequent egg production at all seasons—may be expected properly to indicate the relation of season to egg production. Also, unless the bird has laid some eggs before the test period is begun, and unless healthy, vigorous and actively producing eggs at the end of a year's test, the variations within the yearly record may not reflect seasonal effects alone.

Only birds which fulfilled the above tests were used in this study. The tests were conducted during 15 months, so that a 3-month period at the

end of the test could be united with the first 3-month period to establish the norm for that period or season. Since the average of two periods—taken from successive years—are thus involved it sometimes happens that the tabulation indicates the production of an uneven number of eggs (e.g., 13 = average of 10 and 16) although no "single" or unpaired eggs were laid. From among nearly 50 common pigeons tested we have tabulated the records of the 15 which produced the largest numbers of eggs. Data from a similar group of 15 ring doves were published earlier by Riddle and Honeywell (1924)—before the nature of the seasonal changes in thyroid size was known. A summary of the results thus obtained for these ring doves is again tabulated here.

In the tabulations of this paper the seasonal periods are rather more properly designated by number—first, second, third and fourth—than as fall, winter, spring and summer, with which these numbers nearly correspond. These periods are those fixed in the previous paper on thyroid size, and are continued here exactly as given there. The "first" period includes October, November, December, in the case of common pigeons and ring doves. For "generic hybrids," however, this "first" period includes November, December and January. This grouping of months followed naturally from the monthly values of thyroid size as obtained throughout the year on each kind of pigeon (see Riddle and Fisher, 1925).

In preparing the ovaries for weighing we have uniformly removed from the gland any ovum which had started upon its final period of rapid growth. One such ovum normally increases its weight (within about 108 hours) until it alone weighs from four to seven times as much as all the remainder of the ovary. This type of ovum is detected by the fact that the yolk produced after the initiation of rapid growth is yellow; in the previous stages of slower growth "white" yolk is found. These facts have been discussed in previous papers of this series and elsewhere. In practice this means that before the ovary is weighed all ova exceeding 4 mm. in diameter are separated from it.

Presentation of data. Gonad and thyroid weights are here given for three kinds of pigeons, and these three groups of data must be separately tabulated. The fact that the size of the thyroid changes with age, as shown by Riddle and Fisher, makes it also necessary to divide the data for each kind of pigeon into three or four age groups. Each of these age groups must be further sub-divided into the four seasons, and all these groups must in turn be separated into male and female groups. These repeated divisions of the data result in the inclusion of very small numbers in some of the final sub-divisions; but the large number and nearly uniform character of these final groups supplies adequate evidence for the conclusions drawn. Naturally, however, the groups containing the larger number of measurements are the more significant.

Table 1 records data for males only. It may first be noted that testis size, as well as thyroid size, tends to increase within the tabulated age limits. It is clear therefore that in order to obtain a true measure of the relation of season to either thyroid or testis size the data require subdivision on the basis of age. Although data for thyroids from birds aged 6 to 7 months could be utilized in the study of the seasonal variation of that organ, this age-group can not be utilized in a similar study of the testis. The immature testis of the pigeon is extremely small compared with the mature gland, and variations in the time of attaining maturity do not permit its utilization prior to the beginning of the eighth month.

Examination of the testis weights in "first" and "second" periods (fall and winter) show them to be smaller than in "third" and "fourth" periods (spring and summer) in all of the three kinds of birds. This statement applies to the averages obtained from combining the fall-winter periods into one group, and the spring-summer periods into another. Only one exception is found among the twelve possible comparisons provided in the table; this exception concerns a group (ring doves, 20 to 30 months) in which weights are available for only a single individual in the spring and summer periods. A more compact summary of most of these testis weights (fall and winter combined, and spring and summer combined) is given in table 2. All of those six groups show larger testis size in the six months of spring and summer.

Further evidence for this seasonal difference in testis size is afforded by weights obtained from birds found infested to one or another degree with intestinal worms (*Ascaridia*). These weights are not tabulated here, for reasons pointed out in the paper dealing with thyroid size. There are 190 of these males belonging to 12 groups corresponding to those for healthy birds in table 1. In 10 of the 12 groups the testis size is larger in the spring-summer period; in 2 instances the size is reversed to the extent of 3.5 and 12.8 per cent. The 10 groups having larger testis size in the spring-summer period are larger by from 1 to 88 per cent. The mean percentage of larger size for the 12 groups is 27.

It was not practicable to indicate on table 1 the number of birds supplying data for the four periods of the year, though thyroid and gonad weights are given for each 3-month period. The reader is thus unable to know precisely the average testis weight in the two six-month sections into which the year is divided. The amount of this difference is therefore given here in terms of percentage. Calculating the combined fall-winter weight as 100 per cent, the spring-summer values of the 12 comparisons of table 1 (in order from top to bottom) are larger by the following percentages: 43, 20, 9, 10; 45, 26, 21, 3; 22, 3 (4), 21. Only the single figure given in parentheses indicates a smaller value in the spring-summer period. The mean increase of testis size found for the 12 groups during spring-summer is 18.2 per cent.

TABLE 1

Thyroid and testis size in relation to each other and to season

KIND OF BIRD	AGE GROUP (MONTHS)	NUMBER OF BIRDS (TESTES)	BODY WEIGHT	TESTIS: THYROID	WEIGHTS OF TESTES (GRAMS) AND THYROIDS* (TENTHS OF MGMS.) AT FOUR PERIODS OF YEAR					
					First	Second	Te/Th	Third	Fourth	Te/Th
Common pigeons	7– 8.9	12	355	Tes.	1.228	1.530	33.4	1.777	2.101	72.6
				Thy.	380	445		309	225	
	9–16.9	59	345	Tes.	1.431	1.731	41.1	1.653	2.028	61.1
				Thy.	398	371		256	346	
	17–19.9	12	344	Tes.	1.720	1.825	32.9	1.586	2.382	47.3
				Thy.	408	669		250	588	
	20–30.0	11	377	Tes.	2.045	1.901	34.4	1.451	2.334	31.0
				Thy.	485	661		655	564	
Generic hybrids	7– 8.9	35	159	Tes.	0.692	0.967	54.2	1.346	1.094	83.8
				Thy.	186	120		117	174	
	9–16.9	61	159	Tes.	0.929	1.061	60.9	1.320	1.127	63.9
				Thy.	169	158		145	138	
	17–19.9	19	162	Tes.	1.147	1.199	77.9	1.325	1.388	89.5
				Thy.	137	164		97	206	
	20–30.0	11	169	Tes.	1.105	1.730	70.9	1.213	1.269	100.0
				Thy.	205	195		92	156	
Ring doves	7– 8.9	22	150	Tes.	0.596	0.925	48.6	0.947	0.928	68.2
				Thy.	169	144		132	143	
	9–16.9	50	162	Tes.	1.234	1.206	83.5	1.328	1.089	101.6
				Thy.	145	147		120	118	
	17–19.9	19	169	Tes.	0.994	1.468	84.0		1.040	105.0
				Thy.	137	156			99	
	10–30.0	10	171	Tes.	1.150	1.369	88.7		1.562	96.4
				Thy.	142	142			162	

* Both male and female thyroids are included in the case of the youngest (7–8.9 months) groups.

Similar percentages of increased ovarian size for the six groups of females of table 2 are: 10, 12, 5; 71, 17, 10. The mean spring-summer increase in this case is 20.8 per cent. Measurements were made on somewhat fewer females than males, but all of the six groups yield a similar result. Both

testis and ovary therefore show an increase in weight of approximately 20 per cent over the weight in the fall-winter period. It can be added, however, that it is entirely probable that the seasonal size differences observed in the ovaries are not chiefly differences in the amount of ovarian stroma; but mainly they represent seasonal differences in the amount of white yolk contained in varying *numbers* of oocytes which exceed 1.0 or 2.0 mm. in diameter. Ova exceeding this size are more numerous in the spring-summer period.

The thyroid weights of table 1 show that thyroid size bears an opposite relation to season to that already noted for the gonads. In the six months of fall-winter (first and second periods) the thyroids are larger than those of the following six months in 10 of the 12 possible comparisons. The two exceptions are those for the oldest age groups of common pigeons and ring doves, and these groups are those composed of the smallest numbers (10 and 11) of the table. The more condensed summary for these males given in table 2 shows no exceptions among the six comparisons of these more numerous groups.

The thyroids of the six groups of females are larger in the fall-winter period in five of the six possible comparisons. The exception is found in the age-group (oldest ring doves) with the smallest number (12) of birds. The adequacy of the thyroid data, including the data from birds aged 6 to 7 months and those affected with *Ascaridia*, has been fully treated in the earlier publication to which reference has been made. This point requires no further consideration here.

In table 1 data are given for the ratio of testis size to thyroid size in fall-winter and in spring-summer periods. Examination of those ratios shows that in winter there is a notably larger proportion of thyroid tissue in relation to testis tissue, than in summer. Eleven of the 12 comparisons show this, and the only exception again applies to the smallest group (11 birds) of common pigeons.

The above data show—uniformly in all those age-groups which include larger numbers of individuals—that in both sexes the gonads are smaller during the 6-month period (fall and winter) of larger thyroid size; and that the gonads are larger during the period (spring and summer) of smaller thyroid size. This size difference is both relative and absolute; there is an active and opposite size-change in these two organs at these contrasted seasons of the year. These data apply to captive pigeons given partial protection from cold in winter, and kept otherwise under conditions which in the main permit reproduction to continue throughout the year.

A further point of interest is made clear by the data of table 2. The three kinds of pigeons examined belong to two different zoological families—Columbidae and Peristeridae. The former, represented by common pigeons, are birds of large size—having more than twice the body weight

of the ring doves and the hybrids studied. The ovaries of the smaller species are, however, practically equal to those of the much larger bird. On the other hand, the thyroids of the larger species are more than twice as large as the thyroids of the smaller species. In the common pigeons therefore there is at all seasons a much higher ratio of thyroid:ovary than is found in the smaller species. Corresponding to this fact is the circumstance that common pigeons produce eggs less freely than do ring doves (compare totals of table 3). Here again larger thyroids are associated with restricted egg production.

TABLE 2

A comparison of gonad and thyroid size in Columbidae (common pigeons) with two groups of Peristeridae (ring doves)

KIND OF BIRD	BODY WEIGHT	AVERAGE	SEX	NUMBER	WEIGHT OF GONADS		WEIGHT OF THYROIDS	
					Fall and winter	Spring and summer	Fall and winter	Spring and summer
Aged 9.0 to 16.9 months								
Common pigeons	344	12.0	♂	59	1.513	1.815	367	289
	324	12.1	♀	42	0.338	0.371	436	290
Generic hybrids	159	11.7	♂	58	0.999	1.256	159	142
	161	11.9	♀	58	0.335	0.375	162	159
Ring doves	162	12.0	♂	47	1.218	1.260	146	119
	159	12.0	♀	32	0.317	0.332	141	127
Aged 17.0 to 30 months								
Common pigeons	359	20.7	♂	23	1.828	1.997	577	475
	352	20.4	♀	14	0.315	0.538	470	325
Generic hybrids	164	20.5	♂	27	1.153	1.295	162	151
	163	20.7	♀	15	0.366	0.430	148	142
Ring doves	169	21.1	♂	29	1.157	1.388	141	140
	163	19.5	♀	12	0.299	0.329	139	145

It remains to supply data concerning seasonal changes in ovulation rate in pigeons. Data for the four periods of the year are given in table 3. In only one of the 15 records of common pigeons were more eggs produced during the 6-month period of larger thyroid size; the difference there is small (18:16), and the total of 34 eggs in this record is not close to the maximum. The total yearly egg production of each of the 15 birds listed in the table ranges from 27 to 50 eggs. In the period of increasing thyroid size (October 1 to January 1) these 15 birds produced only 100 eggs;

while in the preceding 3-month period they produced 169 eggs. The ring doves show a still greater discrepancy (107:248) in rate of egg production during these same two periods. The contrast between the records obtained in the winter period (which is a period of large but not of *increasing* thyroid size) and the immediately following spring period (a period of decreasing thyroid size) is less extreme, though it is entirely evident in the

TABLE 3

The ovulation rate during the four seasons of the year as found for the 15 common pigeons having highest rate of ovulation during a 15-month period (earlier summary for 15 ring doves at bottom of table)

NUMBER OF BIRD	NUMBER OF EGGS LAID DURING THE FOUR SEASONS OF THE YEAR							
	First period (October 1 to January 1)		Second period (January 1 to April 1)		Third period (April 1 to July 1)		Fourth period (July 1 to October 1)	
	Total	Singles	Total	Singles	Total	Singles	Total	Singles
1	14	0	8	0	12	0	16	0
2	9	1	11	1	12	0	12	0
3	10	0	8	0	10	0	14	0
4	6	0	8	0	13	0	14	0
5	8	0	10	0	6	0	14	0
6	1	1	9	1	16	0	12	0
7	6	0	9	1	8	0	12	0
8	4	0	10	2	12	0	8	0
9	10	0	8	2	9	1	7	1
10	9	1	3	1	8	0	12	0
11	6	0	8	0	9	1	8	0
12	4	0	7	1	8	0	11	1
13	2	0	4	0	12	0	11	0
14	7	0	4	0	9	1	8	0
15	4	0	6	0	7	1	10	0
Totals	100	3	113	9	151	4	169	2
Per cent "singles"		3.0		8.0		2.6		1.2
Summary for 15 ring doves (earlier published)								
Totals	107	9	219*	9	280	4	248	6
Per cent "singles"		8.4		3.9		2.1		2.4

* Average for two periods forming the beginning (226) and end (211) of the test.

case of both kinds of pigeons. For common pigeons these numbers are, 113:151; for ring doves, 219:280. The reader is referred to the paper of Riddle and Honeywell (1924) for details of the records of the 15 ring doves summarized in table 3. Without exception each bird of that group produced more eggs during the 6-month period which we now recognize as the season of smaller thyroid and larger gonad size.

It seems unnecessary to tabulate further data on the point considered above. The method of selecting the birds for the above tabulations is particularly favorable for showing the maximum ovulation rate of fall and winter periods—because only birds with unusually high total yearly production are included. Our yearly experience with our entire collection of pigeons indicates that at least two to five times as many eggs are laid during May, June and July as in November, December and January. It is not easy, however, to give exact figures on this point; and such figures if presented would be subject to the objection that not all of the birds utilized at one period were also utilized at the other. The general experience of other workers with these animals is also doubtless quite similar to our own. It is common knowledge that in the case of the wild birds of colder climates the period of egg production is strictly limited to spring and summer.

Discussion. The observed size changes in thyroid and gonad would seem to provide a fact of interest to studies on the interrelationships of these incretory organs. In most earlier work on this subject results of a variety of kinds have been interpreted as evidence for an antagonism between thyroid and gonad. Some data, however, indicating synergism not antagonism have been reported. Before the present data can be used for or against either of these interpretations it is necessary to know the meaning of the observed size changes of gonad and thyroid in terms of increased or decreased function of these organs. This point may now be examined.

Both contrasting and parallel changes are here observed to occur in thyroid and gonads. How are the two kinds of change to be regarded? The data from pigeons aged 7 to 30 months show that the gonads and thyroids simultaneously undergo *seasonal* size changes in opposite directions. These same data, however, show that in respect of *age* (within the age limits examined) there is a similar and parallel increase of size in the case of both these organs. Such an increase of size with age is already known to occur in post-puberal stages of the human—at least in the case of the ovary and thyroid. According to Wehefritz (1924) the human ovary increases in size up to 30 to 40 years, and the thyroid probably to 70 years.

The increase of thyroid size with age (in later adult life) probably does not indicate an increase of thyroid-functioning with increased age. We have no data on this point, but if conditions in the pigeon are similar to those in man and mammals this matter is subject to little doubt. In the case of the gonads, particularly of the ovary, the conditions are less simple and probably different. Not only does the actual amount of ovarian and testicular tissue appear to continue to increase beyond the age of 20 months (to 30 or 40 years in the human), but the particular

function of the ovary which has been examined in this paper—the rate of egg production—also apparently continues to increase beyond the age of 20 months. The data bearing on this point can be given only in a complete account of our studies on sex. In general, it must be said that the size changes in thyroid and gonad during advancing age do not seem to contain new facts of unequivocal bearing on the question of antagonism or synergism of these glands.

In the seasonal size changes of the thyroid, however, it is entirely probable that the larger thyroids of autumn and winter are thyroids functioning at a higher level. Some convincing evidence on this point has been considered elsewhere by Riddle and Fisher (1925). On the other hand, the smaller ovaries of autumn and winter have been shown in the present paper to be functioning at a lower level in so far as their function is exhibited by the growth, maturation and liberation of ova. The smaller testes of this same period are also doubtless the expression of a reduced rate of spermatogenesis, since in wild forms spermatogenesis is completely suppressed at this season.

It would therefore at first seem obvious that the alternating or reversed seasonal changes in thyroid and gonad indicate antagonistic relationships between these two glands. Unless accompanied by important reservations I do not think, however, that such a conclusion is either necessary or justified. The reasons are:

First, in birds we are able at many points to distinguish sharply between *sex* and *reproduction*. And there is evidence that both thyroids and gonads in birds are intimately related to both sex and reproduction.

Second, whatever may be said of single or multifarious functions of the thyroid, the gonad is both a reproductive and a sexual organ; and it is only a coincidence of increased thyroid activity with a diminution or suppression of an aspect of *reproduction* that is indicated by our results.

Third, it seems probable that the antagonism involved in this repression of reproduction lies between the thyroid and suprarenal rather than directly between thyroid and gonad.

It has been shown by Riddle (1923) that active growth and dehiscence (ovulation) of ova in the pigeon is definitely coincident with a marked enlargement of the suprarenals. In two earlier papers of this series of studies, Riddle and Honeywell (1924a, 1924b) presented facts which "suggest that ovulation in birds is normally associated with the capacity of the organism temporarily to increase the blood sugar to a value higher than the normal concentration, and that conditions adverse to this capacity tend to suppress ovulation." According to present knowledge a highly functioning thyroid usually effects a diminution of the blood sugar—in this respect opposing an action of the suprarenal. In one of those studies (1924b) it was found that the blood sugar is markedly diminished soon

after the onset of cold weather in the autumn—the precise time at which we now know the thyroids begin to enlarge. In the other study (1924a) it was shown that the blood sugar is depressed, along with a diminution of ovulation, during enforced inactivity or close confinement. Under these last-named conditions it is highly probable that the activity of the thyroid is diminished. It was therefore concluded that "some facts suggest that a decrease of both blood sugar and ovulation rate is sometimes associated with a decreased and sometimes with an increased basal metabolism." If the final part of this statement is a fact, these opposite changes in thyroid activity should probably have different or opposite effects on sexuality though they have like effects on reproduction. A change from the normal, in either direction, causes the diminution or the suppression of a reproductive function, namely, ovulation—thus again illustrating Darwin's observation concerning the sensitiveness of the reproductive functions of wild animals to changes in habitat or conditions of life.

In view of the facts and circumstances just reviewed it seems permissible to conclude only that seasonally increased thyroid activity is in some way opposed to the reproductive processes involved in the production of the definite reproductive cells—ova and sperm. Some light on the mechanism by which this opposed action is effected is to be found in other results on suprarenal and blood sugar changes already reported in this series of papers. The facts do not seem to justify the conclusion that the thyroid and gonad are generally antagonistic; and the data of Korentschewsky (1914) indicate that in certain respects they are synergistic. Korentschewsky's data are concerned with the relations of the thyroid and gonads to protein metabolism and are thus concerned with what I regard as the true *sexual* aspect of thyroid and gonad function.

It remains to consider whether the incidence of season of free egg production and season of diminished thyroid size is mere fortuitious coincidence, or whether the two are causally related. A fairly comprehensive and prolonged study of the size of various organs of the pigeon certainly suggests that this relationship is here intimate and causal. Of the other organs only the thymus of pigeons probably (in wild birds almost certainly) undergoes a profound change (temporary involution) during the winter. But the readiness with which this organ undergoes reduction under many conditions prevents its consideration as an important factor in this connection. Finally, many well-known facts demonstrate that an intimate relationship exists between the gonads, the suprarenals and the thyroids, and this supplies an additional reason for considering the size relationships found here as something other than mere coincidence.

If data for seasonal change in thyroid size were available for a series of wild birds the question just raised could be definitely answered for birds generally. Unfortunately there are no such data on the thyroids of any

bird other than the pigeon. There is, however, the indirect evidence (fully cited by Riddle and Fisher) derivable from the fact that the thyroids of experimental animals generally show increased thyroid activity after fairly prolonged exposure to low temperatures. It therefore seems probable that a similar increase of thyroid function occurs in wild birds exposed to the colder temperatures of autumn and winter. The facts on the other aspects of the problem are definite and conclusive. The gonads, particularly the testes, undergo extreme reduction in wild birds during autumn and winter. This fact is fairly well known, and we shall elsewhere give quantitative data for several wild species of birds. The reduction found in the ovary, however, is here very little compared to that observed in the testis. It is possible, moreover, that the reduction in testis size begins in wild birds immediately after the close of the breeding season and before the onset of cold weather. If this should prove to be the case the existence in these wild forms of some complicating factor (again the suprarenal?) is made probable. In general, however, it is clear that the conditions found in wild birds indicate a relation of season to thyroid size, gonad size and egg production similar to that which has here been found and described in pigeons.

SUMMARY

Data are given showing coincident seasonal changes in the weight of thyroids, testes and ovaries of three kinds of pigeons.

The seasons (autumn and winter) of increased thyroid size are seasons of diminished size of testis and ovary. The actual reduction of gonadal tissue is probably greater in testis than in ovary. The seasons (spring and summer) of decreased thyroid size are seasons of increased size of testis and ovary.

The seasons of free or frequent ovulation coincide with periods of decreased thyroid size and of increased gonad size. The period of restricted ovulation—and of numerous clutches composed of single eggs—coincides with the period of enlargement in the thyroid.

One species of pigeon which has at all seasons a large amount of thyroid tissue in relation to ovarian tissue has a markedly less ovulation or egg producing capacity than have two other species with small thyroids and large ovaries.

These reciprocal size changes do not represent mere coincidence; they probably reflect actual functional relationships of these organs.

The data, however, are not regarded as evidence for a general antagonistic action of thyroid and gonad. Both of these organs exercise functions which are related both to sex and to reproduction. Antagonism is here suggested only between the seasonally enlarged thyroid and a single reproductive function, namely, ovulation and spermatogenesis. This apparent antagonism may rest upon opposed actions of the thyroid and suprarenal.

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THE FREQUENCY OF MOTOR NERVE IMPULSES IN THE CROSSED EXTENSION REFLEX AS SHOWN BY THE ALCOHOL BLOCK METHOD

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INTRODUCTION. The frequency with which nerve impulses are discharged along the motor neurones in sustained reflex and in voluntary contraction of skeletal muscle is a subject about which much has been written and little decided. Some years ago Piper (1) made an extensive study of the electromyogram of the human forearm flexors. He found in the muscular action currents a dominant frequency of about 50 per second; from this observation and a variety of related experiments he was led to the conclusion that this observed rhythm was that of the impulses in the motor neurones innervating the muscles. Buchanan (2), on the contrary, as a result of experiments on frogs, was led to conclude that the observed frequency of action currents in the electromyogram depended on an intrinsic rhythm of response in the muscle, and in no way indicated the frequency of central discharge. Garten (3), Dittler (4) and Beritoff (5) all reported experiments bearing more or less directly on this controversy, and a summary of their arguments has appeared in a previous paper (6). In 1917 Forbes and Rappleye (6) reported the results of experiments similar in principle to those of Buchanan, but dealing, like Piper's observations, with voluntary contractions in man. Chilling the muscles of the hand or forearm by immersion in ice water caused a pronounced slowing of the observed action-current frequency in the electromyogram of voluntary contraction. This result has been confirmed by other observers (in one case unpublished and verbally reported to us) (cf. 7). Since the temperature of the central nervous system was not appreciably altered during these experiments, it was concluded that the observed frequency of muscle action currents did not reflect the true frequency of the motor nerve impulses. It seemed unlikely that the motor neurones, remaining at a constant temperature, should alter their frequency of discharge in consequence of a change in temperature in the muscle. It was recognized that such a change might conceivably result from the cooling of the intramuscular receptors; but this explanation was regarded as improbable. Piper (8) had shown that

muscle responses would correspond with stimuli applied to the motor nerve up to 300 a second. If the muscle can follow so high a frequency of motor nerve impulses, he argued, why should it fail to do so in the case of voluntary innervation? He therefore rejected the view that muscle responded with a rhythm of only 50 per second to nerve impulses of a much higher frequency. Forbes and Rappleye met this argument by a consideration of the effect of high frequency of nerve impulses upon their size and resulting stimulating efficiency. They cited the work of Lucas and Adrian (9), (10), (11) to show that if a nerve impulse occurs early in the relative refractory period following a previous impulse it is subnormal. They also pointed out that muscle fibers have a similar, though longer, relative refractory period in which their excitability is impaired. It was shown that nerve impulses of frequencies up to 250 or 300 per second may be almost or quite full-sized, the intervals sufficing for almost complete recovery. Moreover, the excitability of the muscle fibers has evidently recovered enough in $\frac{1}{300}$ second to enable them to respond to these approximately full-sized nerve impulses. But if the nerve-impulse frequency is much higher than 300 per second the impulses would necessarily be subnormal and therefore unable to excite the muscle fibers until a later stage in the recovery of the latter. In these considerations Forbes and Rappleye found reason for concluding that the frequency of voluntary motor nerve impulses was more than 300 per second, and might be much more than this. They attempted an analysis of the relation between motor nerve impulses and the resulting muscle responses, in which it appeared possible to explain the observed facts by assuming a nerve-impulse frequency of well over 500 per second and a resulting muscle frequency of 50 per second.

If the proposed figures were accepted it would mean that several ineffective nerve impulses reach the muscle during the refractory phase following each muscle response. Subsequent observations reported by Forbes, Ray and Griffith (12) indicate a quantitative revision of the analysis proposed by Forbes and Rappleye. The refractory phase of nerve, using the term to designate the time during which the tissue is unable to respond, was found to be much longer than had been supposed. At mammalian body temperature it is such that the nerve-impulse frequency could not be greater than about 600 per second. Furthermore it was shown (12, p. 589) that in the mammalian nerve-muscle preparation (ankle flexor in the cat) the frequency with which muscles can respond to successive nerve impulses arriving at the maximum possible frequency, differs from this maximum nerve-impulse frequency by far less than was assumed in the analysis of Forbes and Rappleye. It seems clear therefore that, unless the refractory phase of the human forearm flexor muscles is very much longer than that of the tibialis anticus of the

cat, the picture offered by Forbes and Rappleye is wrong in so far as it postulates a nerve-impulse frequency several times as high as that of the muscle responses. It should be noted that the observed frequency in the electromyogram of the interosseous muscle of the hand was considerably higher than in the forearm flexors of the same subject, and that the observed frequency of the electromyogram of the limb muscles of the cat in various reflexes has been found by several observers to be higher than that of either of the human muscles mentioned above. These observed differences may represent actual differences in the refractory periods of the various muscles, and may serve to explain in part the apparent contradiction between the observed frequency in the human electromyograms and the frequency which, in consequence of the various observations on the muscles of the cat, we should expect to result from a high frequency of motor discharge. But, as we shall presently see, there are other reasons for abandoning a view which assumes a uniform and regular rhythm in either motor nerve impulses or muscle responses.

Altenburger (13) has stated, though offering no proof, that the greatest frequency of motor nerve impulses in voluntary contraction is 50 to 60 per second. On this basis he rejects the idea of subnormal motor nerve impulses proposed by Forbes and Rappleye, maintaining that it would require a refractory period of 0.02 second in nerve. From what we have just said it will be clear enough that this argument rests on a complete misunderstanding of the discussion of Forbes and Rappleye.

In studying the electromyogram it is a question of some importance whether we should count every excursion of the string revealed in the record, or whether we should heed only the dominant rhythm of the major excursions. In a recent paper, Forbes and Cattell (14) have shown that the frequency found by counting every visible excursion in an electromyogram of the crossed extension reflex depends chiefly on the mass of the string used for recording, for the electromyogram at best is highly irregular, apparently indicating that now many, now few fibers are responding together. The number of oscillations discernible in the record probably depends on how small a group of approximately synchronous individual action currents the string can reveal with the definition rendered available by the optical system of the recording device. Further, since cooling the muscle fibers increases the duration of their action currents, we may expect it to increase the overlapping of responses in different groups of fibers. In this way the responses of two groups of fibers which at high temperature would appear distinct might be apparently merged into one at the lower temperature, although the interval between the beginnings of the responses of the two groups remained the same. Thus when every excursion of the string is counted, an apparent change in frequency might be found when no real change existed. This considera-

tion may account for part of the change recorded by Forbes and Rappleye under the heading "total" in tables 1 and 2 of their paper, but it is hard to see how this consideration could explain the pronounced change in the dominant rhythm which was also apparent in their records.

During the last three years Adrian and Olmsted (15) and Cooper and Adrian (7), (16), (17) have reported many significant experiments bearing on this problem. The conclusions of Adrian and Olmsted have already been discussed in a previous paper (12). The observations upon which their conclusions are based have, moreover, to a considerable extent been superseded by the more recent observations of Cooper and Adrian (16) in which an improvement was made in the technique of recording. Cooper and Adrian, first in the case of the frog (7) and later in the case of the cat (16), were led to the conclusion that, in the flexion reflex at least, the observed frequency in the electromyogram corresponds with the true frequency of motor nerve impulses, a view which harmonizes with Piper's original interpretation. This conclusion has received additional support from the work of Gasser and Newcomer (18) on the phrenic nerve and the diaphragm; yet it is conceivable that in the case of this particular mechanism there is a different relation between nerve impulse and muscle response from that found in the case of the skeletal muscles of the limbs. It seems to us that some of the conclusions drawn by Cooper and Adrian (16) do not necessarily follow from the data which they present. The reason for this we shall defer until the discussion following the description of our results.

Since much of the evidence cited in connection with this discussion is derived from observations on both the flexion reflex and the crossed extension reflex, we should, before going further, note the difference between these reflexes. Liddell and Sherrington (19) with mechanical, and Cooper and Adrian (17) with electrical registration, have compared these two reflexes and brought out important differences, as follows: *a*, in the flexion reflex a single volley of afferent impulses evokes at once a response in all or nearly all of the motor neurones of the reflex arc; in the crossed extension reflex, on the other hand, very few of the motor neurones are called into action by the first volley of afferent impulses, but, as repeated volleys reach the center, the discharge of motor nerve impulses becomes more and more general; in other words, the development of a maximum of reflex activity is slow and gradual instead of immediate and abrupt. *b*, Corresponding with this difference there is an enormously greater after-discharge in the case of the crossed extension reflex. It has been said (17, p. 245, etc.) that the flexion reflex arc acts more like a nerve-muscle preparation than the arc of the crossed extension reflex. On the other hand, Sherrington (20) has shown that even in the low spinal preparation, and even when evoked by a single

induction shock, the flexion reflex normally shows an appreciable after-discharge (cf. 21). Cooper and Adrian found the after-discharge in this reflex to be much greater in the decerebrate preparation than in the spinal animal. The observations of Forbes, Cobb and Cattell (22) on the flexion reflex support these findings, and show that in the decerebrate preparation without spinal transection the repetition of afferent stimuli causes a large increase in after-discharge. *c*, In the case of rapidly repeated afferent stimuli, Cooper and Adrian (16) have detected in the electromyogram of the flexion reflex a rhythm corresponding with stimulation up to frequencies of 250 to 300 a second. In the case of the crossed extension reflex, any such regular correspondence was much less easy to detect and in some cases was not found at all (17).

In seeking a reflex which when artificially evoked shall resemble as nearly as possible sustained voluntary contraction in the manner of its motor-neurone discharge, we have turned to the crossed extension reflex, in which the motor neurones are less directly accessible to the afferent fibers, and which is accordingly characterized by a longer latency, a more sustained type of response and a less close correspondence between the rhythm of muscle response and that of the stimuli.

All of the inferences in the literature as to the frequency of motor-neurone discharge in sustained contractions of central origin have been made from the study of action-current rhythm in the muscle. It is desirable, if possible, to attack the problem by a more direct method; that is, by determining what goes on in the motor nerve. An attempt to do this in the case of the crossed extension reflex by the most direct possible method, namely, recording the action currents of the motor nerve itself, has been reported by Forbes and Cattell (14). Even with the amplification obtained with the electron tube and with a string of very small inertia they were unable to obtain any record of action currents after the sustained discharge of impulses in this reflex had become established. The reason for this probably lies in the fact that the individual action currents are of extremely brief duration, and that when the discharge of impulses becomes general practically no two motor neurones are discharging impulses exactly in phase with each other. With complete overlapping of individual impulses the galvanometer fails to record anything.

There is, however, another method of attacking the problem, less direct, yet more so than the inferences drawn from action currents in the muscle. This method was devised by Adrian and Lucas (10, p. 93) for studying certain problems of conduction in the nerve-muscle preparation. It consists in establishing with alcohol a partial block in the course of the motor nerve. This partial block was designated by Lucas a "region of decrement," but the recent work of Kato and his collaborators (36),

confirmed by recent observations in this laboratory (37), has forced us to revise our conception of the nature of such a block. The fact still remains, as shown by Adrian and Lucas, that a partial block will stop the impulses of subnormal magnitude occurring during the relative refractory phase following a previous impulse, yet will fail to stop the full-sized impulse set up in the nerve after a period of rest. When such a block is applied to a motor nerve it is, therefore, possible to determine whether impulses traversing it are subnormal or not. In view of the all-or-none principle, it is well established that a subnormal impulse only occurs in normal nerve during the relative refractory phase following a previous impulse. The duration of relative and absolute refractory phase can be measured by direct experiment, and in this way it is possible to determine approximately the interval elapsing between impulses, if they can be shown to be stopped by a partial block which nevertheless can still be traversed by a full-sized impulse.

METHOD. In accordance with the principle just outlined, our plan of attack on this problem was to establish a partial block in the course of a motor nerve involved in the crossed extension reflex, then to evoke this reflex from time to time as the block became more nearly complete. There were two possible results; the reflex response might disappear in consequence of a partial block, which would signify that the motor nerve impulses involved in it were of subnormal magnitude, and therefore were passing so frequently that each occurred during the relative refractory period following its predecessor; or the reflex response might persist until the block became complete, which would prove a longer interval between impulses. It was necessary by way of control to determine the completeness of the block at frequent intervals during the experiment by testing its power to stop full-sized impulses set up in the motor nerve by direct stimulation. Finally, in order to determine the interval between impulses which blocking, in the case of the reflex, would signify, it was necessary to find by direct experiment the interval between two motor nerve impulses at which the second would just fail to pass a similar block. This briefly is the outline of our experimental method. The details of procedure follow.

In all experiments cats were used, decerebrated under deep ether narcosis, transection being at the level of the anterior colliculi. In all experiments with the crossed extension reflex we made our observations of reflex response on the gastrocnemius muscle and applied the block to its motor nerve, the popliteal branch of the sciatic. The reflex was evoked by stimulating the sciatic nerve in the opposite leg. Stimuli were also applied to the motor nerve proximal to the block for the purpose of control. The arrangement is shown diagrammatically in figure 1.

The block was produced by narcotizing a short length of the nerve.

A small, hard-rubber cup, with slots by which the nerve could be led through the middle and thereby immersed in a narcotic solution, was applied; this was precisely like those described by Olmsted and Warner (23). The inside diameter of the cup was 10 mm. The solution employed was one of alcohol (13 to 16 per cent) in mammalian Ringer solution. When we applied the cup to the nerve the slots were made watertight with vaseline and a mica cover was applied to enclose the fluid

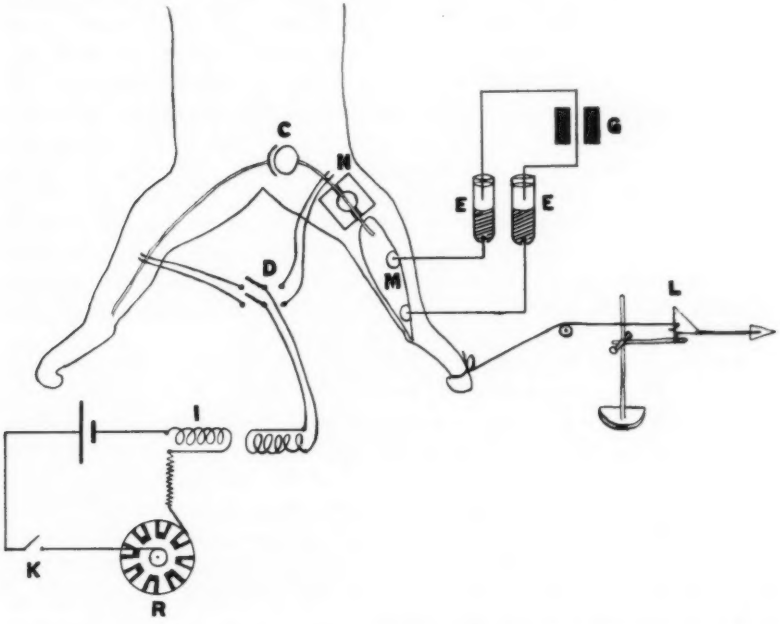

Fig. 1. Diagram to show arrangement of preparation and apparatus. *I*, inductorium. *K*, key in primary circuit for single shocks. *R*, rotary interrupter for tetanizing stimuli. *D*, double-pole double-throw switch to shift stimulation from afferent to motor nerve. *C*, spinal center. *N*, narcotizing cup on motor nerve. *M*, gastrocnemius muscle. *E*,*E*, agar electrodes. *G*, string galganometer. *L* muscle lever for recording on kymograph.

completely. In one test the block was established by wrapping around the nerve absorbent cotton soaked in the alcohol solution.

Since it is well established that proprioceptive impulses arising in the extensor muscles themselves are essential to the state of decerebrate rigidity (24), and influence to a considerable extent the crossed extension reflex evoked in the muscles from which they arise, and since these impulses, to reach the center, must pass through the block, there is a possi-

ble source of confusion if the block abolishes these proprioceptive impulses more readily than the motor impulses. We might find a marked reduction in the reflex contraction, resulting from the block, and suggesting that this had stopped many of the motor impulses, yet the effect might in reality be due to the blocking of the afferent impulses from the muscle and the consequent withdrawal of their reinforcing influence upon the development of the reflex.

To obviate this source of error we performed some of our experiments on de-afferent preparations, in which the dorsal roots of the sciatic nerve on the side of the observed muscle were severed before we made the observations. Some experiments were made on preparations with the afferent paths intact, for it is well established that the crossed extension reflex can occur in the de-afferent animal (25), and therefore does not depend necessarily on proprioceptive impulses. We should expect clearer evidence from the de-afferent preparation. But if in the others the reflex should be abolished by the partial block on the motor nerve, or if not wholly abolished, far more reduced than could be expected from the mere blocking of proprioceptive impulses, this would be evidence of the blocking of motor impulses. Therefore we felt that we might also obtain significant data from the intact preparation.

Another reason for using the de-afferent preparation is that otherwise there will be a certain amount of decerebrate rigidity in the muscle under observation and this involves motor nerve impulses in some of the fibers of the popliteal nerve. Consequently the stimulus applied to the nerve will find some fibers in either the absolute or the relative refractory phase (26); those fibers in the relative refractory phase will respond with subnormal impulses instead of the full-sized impulses which should furnish the basis of our comparison. The result of this effect will be to minimize the contrast, if there be such, between the reduction of responses to reflex and to motor nerve stimulation.

In two experiments we tried severing the afferent roots after decerebration and immediately before proceeding with the experiment. These experiments were not successful, for in one case the animal died immediately after de-afferentation, and in the other the reflex was too feeble to yield satisfactory results, although with the aid of strychnine we obtained small reflex contractions which served for a meager test. As mentioned in a previous paper (14, p. 147), in which these experiments were referred to in connection with another problem, we ascribe this failure of the reflex to the disturbance of the spinal cord in the operation of severing the dorsal roots. Since this method was unsuccessful, we resorted to the plan of severing these roots aseptically several days before the experiment. Four cats were thus prepared from five to eight days in advance. Under ether anesthesia laminectomy was performed, the dura opened,

and the dorsal roots of the last two lumbar and the first sacral nerves on the right side severed.

In all experiments, after decerebration the left sciatic nerve was cut in the popliteal space and Sherrington shielded electrodes applied to the central end, so connected with an inductorium as to deliver ascending break shocks. Through a long incision in the right thigh the sciatic nerve was exposed from hip to knee, and its peroneal and popliteal branches carefully dissected apart. The peroneal was cut at the hip to get it out of the way. In the last three experiments the anterior crural and hamstring nerves were cut, in order to eliminate disturbing mechanical action which might arise from the muscles innervated by them; and, for the same reason, in the last two experiments (June 8 and 9) the tibial branch of the popliteal was cut beyond the point where the gastrocnemius branches are given off. The femur was rigidly clamped to a stand, and the lower end of the tibia was clamped as firmly as possible without interfering with the freedom of motion of the Achilles tendon. A pair of shielded electrodes was applied to the popliteal nerve in the upper thigh, in order to control the experiment by testing the response to motor nerve stimulation in alternation with the reflex. The glass shield for these electrodes was similar to that on the left sciatic nerve, except for a slot cut in the side to render it applicable to the uncut nerve. It was applied with great care to avoid stretching or jamming the nerve, and secured in place by sutures in the adjacent tissues. The alcohol block, described above, was applied to the popliteal nerve 2 or 3 cm. distal to these stimulating electrodes.

Contraction of the gastrocnemius muscle was recorded by tying one end of a thread to the foot and the other end to a light aluminum bell-crank lever writing on a smoked drum, its motion being somewhat restrained by the tension of an elastic rubber band. Time was also recorded on the drum by means of a Jacquet clock.

In the majority of experiments we recorded the action currents of the gastrocnemius muscle simultaneously with the mechanical record, by means of a Hindle string galvanometer. The technique of this procedure has been described in previous papers (27), (14). The electrodes consisted of silver wires coated electrolytically with silver chloride dipping into Ringer solution in tubes containing a jelly made of agar-agar and Ringer solution. In the lower end of each of these was embedded a wick of twine kept wet with Ringer and secured in contact with the muscle by a suture through the fascia. Only small openings were cut in the skin to admit the wicks.

When all the above preparations had been made, we began by recording the reflex contraction of the muscle evoked by stimulating the opposite sciatic nerve, then a series of contractions in response to single stimuli

applied to the motor nerve. The reflex stimuli were applied with a rotary interrupter, previously described (28), (29), interrupting the primary current from 40 to 50 times a second, and the coil distance was such that the make shocks were subminimal and the break shocks were presumably maximal with respect to the afferent nerve (see 30, fig. 1), and evoked a vigorous reflex contraction. Sometimes tetanizing stimuli were also applied to the motor nerve, as well as single stimuli, in order to compare the maximal tetanic contraction thus obtained with the reflex contraction.

After taking several records of both the reflex response and that obtained by maximal motor nerve stimulation with the motor nerve undisturbed, but with the empty cup in place, we introduced the alcohol solution into the cup, and continued the alternate motor nerve and reflex stimulations as the block became more and more complete, until no response followed either stimulus. Then the alcohol was removed and the nerve washed with Ringer solution and the same alternation of stimulation was continued until recovery was complete. After full recovery the alcohol block was usually applied again and the experiment repeated. With one preparation the experiment was performed five times.

In choosing the strength of alcohol we sought to establish a complete block as slowly as possible. If the alcohol was too weak the block never became complete; if too strong, the block developed so rapidly that it was difficult to compare reflex and motor nerve stimulation on equal terms, for the degree of block would change materially between successive tests, however quickly they were made. These considerations narrowed down the concentrations at which results could be obtained to between 13 and 16 per cent. The fact that this is so much stronger than that which has been used in blocking experiments with frog nerves may be due in part to the continuance of a small blood supply in the mammalian nerve in situ, which dilutes the alcohol slightly as the experiment proceeds. Evidence of this was found in some experiments in the presence of blood clots on the nerve within the narcotizing chamber.

In the control experiments whereby we sought to evaluate our data by stimulating a motor nerve at chosen intervals and finding the interval at which a second nerve impulse failed to pass the block, we used a Lucas pendulum. This apparatus, whereby the interval between the break shocks from two coils can be adjusted to within 0.05σ, has been described in a previous paper (12, p. 559). In a decerebrate cat the peroneal nerve was dissected from the popliteal, severed at the hip, and shielded stimulating electrodes were applied. The contraction of the tibialis anticus muscle was recorded by means of a thread tied to the foot. This nerve-muscle preparation was used instead of the popliteal and gastrocnemius since the peroneal nerve is the more accessible. The inside diameter of the cup used in the control experiments was 8 mm.

The criterion for the passage of the second nerve impulse through the block is summated contraction (see (31), (32, p. 31)). This criterion is available except in those few cases in which the second impulse arrives too early in the refractory phase of the muscle to excite it again. By selecting an interval such that summated contraction occurs in absence of the block, we may be sure that when the block abolishes summated contraction it has stopped the second impulse. The procedure, then, was to record on a stationary drum the maximal contraction in response to a single stimulus, that is, the simple twitch of all the fibers; then to set the pendulum for delivering two stimuli at an interval of more than 1.7σ and less than 3σ (see 12, p. 572), i.e., such that the second impulse, starting in the relative refractory period of the nerve, would be subnormal; then to choose a strength of second stimulus which would give maximal summation, i.e., would set up a second impulse in all the fibers of the nerve, and record the resulting amount of summated contraction. The block was then applied between the point of stimulation and the muscle, exactly as in the case of the reflex experiment, the concentration of alcohol varying from 10 to 16 per cent in the different experiments. At frequent intervals the contractions in response to single and double stimuli were recorded in alternation. As the narcosis deepens a decrease in the maximal contraction in response to a single stimulus would signify that in some fibers the block has become complete, i.e., will stop a full-sized impulse. A decrease in the summated contraction in response to two stimuli at a time when the single maximal contraction has shown no such decrease would signify that in some fibers there is a partial block, i.e., that subnormal impulses are stopped, although full-sized impulses are not. Complete disappearance of summation on double stimulation while contraction still results from the single stimulus, would signify that no subnormal impulses can pass the block, although in some fibers, at least, full-sized impulses will still get through.

Since summated contraction is the essential criterion in this method, and since the phenomenon has been misconstrued as a result of a misunderstanding of the all-or-none law of muscle contraction, it seems well, before proceeding, to endeavor to clear up the misunderstanding. It has been argued (33) that in order to reconcile with the all-or-none law summated contraction in response to repeated stimuli, we must suppose that the first stimulus, though "maximal," failed to excite all the muscle fibers, and that the second stimulus has excited some which did not respond to the first. This argument is based on a misunderstanding which has already been discussed in a previous paper (14), but a clear understanding is so essential to our problem that a brief explanation here is desirable. First we should distinguish between the contractile twitch and the propagated disturbance which underlies it. The duration of

even the briefest contraction is so long compared with that of the propagated disturbance and its ensuing refractory phase that an early second response can occur long before the shortening has reached its height. The second release of energy, therefore, is added to the first, and the augmented mechanical effect appears as a greater contraction. In short, two or more functional responses may produce perfectly fused twitches —apparently a single contraction. The all-or-none law does not state that the shortening or the tension in an active muscle fiber must be the same under all conditions; it states that the size of the individual response, whether measured by the resulting contractile tension (34) or by the action current (35), is independent of the strength of stimulus. This has been demonstrated by single stimuli. If a second stimulus is applied while the contraction in response to the first is still in progress, an increment of contractile tension appears as increased shortening, but this increment also obeys the all-or-none law.

Boycott (31) and Lucas (10), when they used summated contraction as the criterion of a second response, showed clearly enough that they recognized the capacity of the individual fiber to contract more on repeated stimulation than it could in the simple twitch. In so doing Lucas implied that his first stimulus, being maximal, had excited all the fibers of the muscle, and therefore summated contraction was proof of double response.

Results. *Control experiments.* In describing the results, we shall begin with those of the control experiments in which the block was tested by means of double stimuli applied directly to the motor nerve. Experiments of this type were performed on five different preparations. They showed strikingly wide variation as regards the relative effects of alcohol on full-sized and subnormal impulses. As stated in the last section, a reduction in the amount of summation on double stimulation occurring at an early stage of narcosis, at which the single maximal twitch has shown no decrease, is evidence that the second (subnormal) impulses are blocked in some fibers, although full-sized impulses can still pass.

In the papers of Lucas and Adrian dealing with alcohol narcosis of amphibian nerves, it has generally been assumed that the narcotic diffused rapidly into the nerve trunk and affected all the constituent fibers almost simultaneously, and indeed their results appear to justify this assumption. In our experiments, on the other hand, we find indications that the blocking is far from simultaneous in all the fibers of the nerve. There is no doubt that in a given fiber a subnormal impulse may be extinguished by a narcotic block which is yet unable to stop a full-sized impulse. This fact stands out clearly in the amphibian experiments just cited; and there is, moreover, abundant evidence of it in our experiments with mammalian nerves. If all the fibers in a nerve were affected

equally, a second impulse set up at any time during the relative refractory period would be blocked in every fiber before the first impulse was blocked in any fiber; therefore we should expect summated contraction to disappear completely before the size of the maximal single twitch was reduced at all, provided the second stimulus was applied during the relative refractory period. In only one of our preparations was there an approximation to this condition. In all cases the size of the single twitch fell off before summation at even the briefest interval had wholly disappeared, in fact even before summation was appreciably reduced. In other words, some fibers had ceased to conduct full-sized impulses through the block before all, or even many of the fibers had ceased to conduct even the earliest (and therefore smallest) subnormal impulses. In one preparation the unequal action of the narcotic was so extreme that the summated contractions continued to show their original relative excess over the single contractions almost till contraction ceased altogether, although the stimulus interval was only 1.8σ, and the response interval presumably little greater, and therefore the second impulse about as small as it is possible to set up (12).

The progress of narcosis in this case is shown in figure 2. In the early stages it is impossible to judge from the tracing whether in *any* fibers, still conducting full-sized impulses, the subnormal impulses had been stopped, but clearly there could at most have been few such fibers. In the majority, almost as soon as the subnormal impulses were stopped, conduction must have ceased altogether. But just before the contractions ceased, thereby showing total block in all the nerve fibers, summation almost disappeared, showing that of the few fibers not wholly blocked scarcely any could conduct these early second impulses. Thus even this preparation showed a differential effect of the block upon subnormal impulses.

All the other preparations showed this more distinctly. In two out of the five, by the time the simple twitch, as measured on the drum, had fallen off to between 50 and 60 per cent of its original size, summation was no longer found with the briefest stimulus interval, and to obtain summation again it was necessary to increase this interval to 2.4σ in one preparation, and to 3σ in the other. In the latter, when the single twitch had fallen to 25 per cent of normal it was impossible to evoke summation even with a stimulus interval of 3σ. Figure 3 shows groups of responses to stimuli, alternately single and double, in this experiment, illustrating various stages in the disappearance of summation.

In the two other preparations we found conditions intermediate between those shown in figures 2 and 3 respectively. In one, after 27 minutes of narcosis with 14 per cent alcohol, the myograph of the single twitch showing 10 per cent of its original value, summation had disap-

peared with a stimulus interval of 1.8σ, but was still found at 2σ. In the remaining preparation stimuli at an interval of 0.8σ, presumably evoking nerve impulses separated by about 1.8σ (12, p. 572), caused

Fig. 2. Kymograph record showing summation persisting as long as single twitch, signifying unequal narcotization of the fibers in the motor nerve. October 16, 1924. In this and all other drum records a rise in the myograph line indicates contraction of the gastrocnemius muscle. The drum was stationary during each contraction. Each group of 5 or 7 contractions was recorded in fairly rapid sequence, about half a minute being occupied in a series. In each group the first contraction was evoked by a single maximal stimulus, and thereafter single and double stimuli were applied in alternation. The second stimulus was strong enough to evoke maximal summation. The successive groups were begun at the following intervals after the block was applied: 1 m. 45 s.; 3 m. 5 s.; 4 m. 8 s.; 5 m. 15 s.; 6 m. 25 s.; 8 m. 40 s.; 9 m. 30 s.; 10 m. 30 s.; 11 m. 5 s.

Alcohol solution, 16 per cent. Stimulus interval in all 1.8 σ. First stimulus 17 Z in all (least maximal value, 8 Z) (coreless coil). Second stimulus 16 Z in all (least maximal value, 10 Z).

pronounced summation till the single twitch had fallen to 20 per cent of normal. At that point summation became very slight, but persisted appreciably till the block was complete.

The longest stimulus interval at which summation failed, while conduction of full-sized impulses persisted, was 4σ, and in this case contraction had almost ceased altogether. The temperature of this preparation was 30°C.; at normal body temperature it is safe to assume that even at 3.5σ after a previous impulse the nerve would have quite recovered its ability to conduct a full-sized impulse (cf. (38), (12, p. 586)). We may therefore conclude that any evidence of differential blocking in the animal at normal temperature (or within 2° or 3° of it) would signify that the

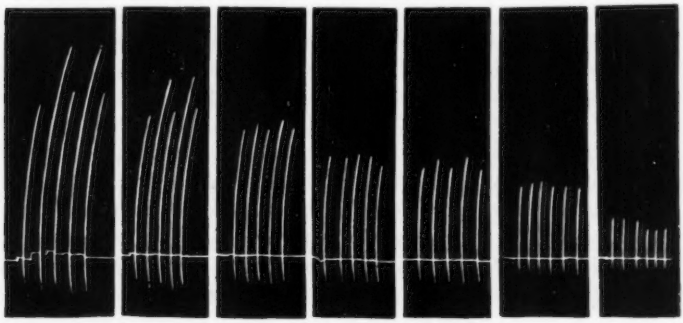

Fig. 3. Records showing disappearance of summation before single twitch, signifying selective action of block on subnormal nerve impulses. February 19, 1924.

Procedure the same as in figure 2. First group before narcotization. Times of remaining groups after block was applied: 14 m. 0 s.; 22 m. 0 s.; 25 m. 0 s.; 27 m. 15 s.; 39 m. 0 s.; 42 m. 30 s.

Stimulus interval, 2σ in groups 1, 2, 3, 4 and 6; 3σ in groups 5 and 7. The stimuli were between 60 and 250 per cent more than the least maximal value.

blocked impulses had followed their predecessors by not more than 3.5σ, and probably by less than 3σ.

In these control experiments the time required to reduce the size of the simple twitch (i.e., totally block some nerve fibers) varied from 2 to 15 minutes. Total blocking of all fibers required from 10 to 34 minutes.

Just why our mammalian experiments should have so differed from the amphibian experiments cited above in the development of complete block in some fibers before partial block could be detected in others is not clear, nor is it clear why there should have been so much difference between individual experiments in this respect. The greater thickness of the mammalian nerve and variations in its blood supply may have been contributing factors. Carefully controlled quantitative work might

show that the difference between individual experiments depended largely on the concentration of the alcohol or on the length of the region of the nerve subjected to its action, or on both. Working within the leg of the animal it was somewhat difficult to limit contact of the alcohol solution strictly to the region within the cup, for sometimes the alcohol leaked out through the slot. In this connection the important fact is that the manner of application of the narcotic block was as nearly the same as that used in our reflex experiments as we were able to make it, excepting the slight difference in the size of the cups.

This brings us to the question of the significance of these control experiments in the interpretation of those performed on the crossed extension reflex. We must expect greater difficulty in detecting evidence of subnormal impulses in the reflex if the component fibers of the motor nerve are unequally acted on than if they were all narcotized alike. Our method of attack is to compare the effect of the block upon reflex motor impulses (whose degree of subnormality we wish to determine) with its effect on full-sized impulses artificially induced. This condition in which the individual fiber progresses from partial block to total block rapidly, while the involvement of increasing numbers of fibers proceeds slowly, militates against a clear differentiation between subnormal and full-sized impulses. For in this case the responses to reflex and to motor nerve stimulation will fall off simultaneously, as the block involves more fibers, whether the reflex impulses are subnormal or not. On the other hand if a difference in the action of the block does appear between the responses to motor nerve and reflex stimulation, its significance as to the subnormality of the reflex impulses becomes all the greater. At all events, our control experiments show in most cases enough differentiation between subnormal and full-sized impulses to offer a fair prospect of blocking the reflex response, if this consists wholly of subnormal impulses, before the block shall have become complete. And if in none of our reflex experiments do we find any difference at all between the falling off of the reflex response and that of the response to motor nerve stimulation, it will be strong evidence that practically all the motor nerve impulses involved in the reflex are full-sized. With these considerations in mind let us examine the results of the reflex experiments.

Observations with the crossed extension reflex. In addition to the anticipated difficulty resulting from unequal narcosis of component fibers in the nerve trunk, we encountered certain other minor difficulties in interpreting our results. In the first place the afferent stimuli which evoked a strong crossed extension reflex, also evoked vigorous contraction of the muscles of the trunk and fore limbs. These, of course, persisted throughout the blocking of the nerve to the gastrocnemius muscle. Although the femur and tibia were clamped as rigidly as was mechanically feasible,

it was impossible to prevent a slight disturbance from these other muscles being transmitted to the writing lever; therefore, even after the gastrocnemius muscle was totally paralyzed by the block we sometimes found in the record what appeared to be a small residual contraction. It was almost always possible, however, to determine by careful inspection of the limb whether or not the gastrocnemius muscle was really contracting. These observations were carefully noted and served on the whole as a satisfactory check, enabling us to discount the small mechanical disturbance which proved irrelevant.

Another difficulty in the way of estimating the comparative decline of responses to reflex and motor-nerve stimulation, as the narcotic involved increasing numbers of fibers, was uncertainty as to the precise nature of the correlation between amount of contraction and number of fibers involved in its production. A given percentage decline in the twitch resulting from a single stimulus and an equal percentage decline in the sustained contraction resulting from the reflex might not signify the blocking of the same percentage of all the nerve fibers involved in the two cases, for there is a mechanical difference between a twitch and a sustained contraction. A maximal single twitch cannot shorten a muscle as much as a complete tetanus, nor even as much as a sustained reflex contraction which apparently involves the response of comparatively few fibers at a time (14, fig. 13). If, to make the mechanical conditions more closely comparable, tetanizing stimuli were applied to the motor nerve, it might conceivably cause approximately full extension of the ankle joint, even after a considerable proportion of the fibers had been blocked, whereas the reflex even at its maximum, being rarely if ever a complete tetanus of all the muscle fibers, might be visibly reduced by the blocking of a smaller percentage of the motor nerve fibers involved in maintaining it. In view of these uncertainties we cannot hope for extreme accuracy in estimating the percentage of fibers blocked in any observation; yet if there is a gross decline of the reflex response over a period of progressive narcosis during which there is little or no decline in the maximal twitch from motor-nerve stimulation, since this maximal twitch does not cause full extension of the ankle, we may certainly look on such a result as significant in showing a selective blocking of motor nerve impulses involved in the reflex.

In the case of electrical records a similar uncertainty as to the significance of a given percentage decline in the galvanometric excursions renders the interpretation difficult, especially since in the case of the reflex response, the probability of the action currents of the individual fibers being often out of phase with each other, greatly complicates the problem. Yet the records, properly interpreted, may be expected to show significant data.

We must also expect to find a small electrical artefact (escape of stimulating current) in the galvanometric records, and must exercise care in distinguishing this from the action currents of a small number of fibers when the block is almost complete. We must also be on the lookout for the possible appearance of the action currents of some other muscles, conducted through the tissues into the galvanometer circuit.

For brevity we shall designate the maximal single contraction in response to motor nerve stimulation the "neuromyal twitch" (39). In eight preparations, de-afferent and otherwise, we made successful records during twenty-two separate narcotizations of the motor nerve. In all of these a differential effect of some sort appeared; that is, the reflex response, or at least some part of it, showed during the progress of narcosis a reduction in which the neuromyal twitch did not share. The results were not quantitatively uniform; in most cases the reflex contractions disappeared before the block was complete, i.e., while motor nerve stimulation was still able to evoke contractions. But in one preparation during two narcotizations small reflex contractions persisted as long as the neuromyal twitch, that is, until the block became complete; in another the reflex persisted almost as long as the neuromyal twitch. Yet even in these cases there were, at certain stages in the progress of narcosis, conspicuous changes in the reflex contractions not correlated with comparable changes in the neuromyal twitch. Even if the maximum of reflex contraction changed no more than did the corresponding neuromyal twitch, either its speed of onset or the amount of after-discharge or both showed a marked decline,—more than could be explained by the number of nerve fibers which the neuromyal twitch showed to be totally blocked.

Let us examine these results in more detail,—first the mechanical records and then the electromyograms, and in each case first the de-afferent preparations and then those with the afferent roots intact.

In the three de-afferent preparations which yielded results we obtained records during the establishment of the block in ten narcotizations. Measurements of the drum records of the neuromyal twitch in each of these showed the following results: In one preparation, the reflex contractions disappeared in the four successive narcotizations when the neuromyal twitch was reduced to 41, 25, 14 and 13 per cent of its normal size. The maximal contraction obtained before each successive narcosis is taken as 100 per cent in each case, since a permanent block in some fibers or an accidental alteration of the mechanical conditions might vitiate comparisons if the initial height of contraction at the beginning of the whole experiment were taken as normal. In the next preparation the percentages of neuromyal twitch, when reflex contraction ceased, in three successive tests, were 0, 0 and 29 per cent. This was the experiment in which, as already stated, some reflex contraction persisted twice

until the block became complete; but even in this preparation the third narcotization showed a clear differential effect. In the third de-afferent preparation the first narcotization was not carried far enough to block the reflex completely, but the reflex contractions were already almost gone when the neuromyal twitch was only reduced to 71 per cent of its original height. In the next two narcotizations the reflex contractions ceased when the neuromyal twitch was reduced to 27 and 39 per cent respectively of its value before narcosis.

Analyzing the data in a different way we compared the time from the application of the narcotic to the first decline of contraction in the case of the reflex and the neuromyal twitch. These figures are not very satisfactory, since fortuitous fluctuations in the amount of reflex contraction render difficult the detection of the first significant decline, and at best the time of decline can only be fixed to the nearest quarter minute, as this was the least interval that was feasible between tests. But the collected data shown in table 1, in which the true beginning of the decline was estimated as nearly as possible in each case admitting of such measurements, will prove significant in showing the predominant action of the block upon the reflex response.

A better idea of the differential blocking of reflex motor nerve impulses can be had by inspection of the drum tracings which are reproduced in figures 4 and 5. In figure 4 is reproduced the entire course of narcotization in one of the two tests mentioned above, in which some reflex contraction persisted as long as the neuromyal twitch. Even in this case it should be noted that for a time just before the middle of the record there is a marked decline in the reflex while the neuromyal twitches are continuing with almost constant magnitude. We may infer from this that during that part of the narcotization many more reflex impulses than "direct" impulses were succumbing to the block. It may also be seen by comparing the *duration* of reflex contraction with that of stimulation, as shown by the signal magnet record, that the reflex after-discharge was greatly reduced as the block developed. This reduction of after-discharge appeared early in the narcotization and was relatively far greater than the reduction of the actual height of contraction. It was so great as to indicate that the motor nerve impulses involved in the after-discharge were in very large measure stopped by the block when a considerable percentage of the nerve fibers were still able to conduct the full-sized impulses set up by direct stimulation. Other records, made from this and other similar preparations, showed an equally striking reduction in the speed of onset of reflex contraction as the block developed. Although the ultimate shortening of the muscle during a prolonged reflex response was not much more reduced by the block than was the neuromyal twitch, yet the beginning of the reflex response became strikingly sluggish. Thus

in the early stages of the reflex, as well as in the after-discharge, there seemed to be a differential effect of the block upon reflex motor impulses as compared with those evoked by direct stimulation.

Figure 5 shows selected tracings from the record of another de-afferent preparation, contrasting in each case the condition when the narcotic had just been applied, i.e., before it had exerted any appreciable effect,

TABLE 1

	DATE	CONCENTRATION OF ALCOHOL	TIME FROM APPLICATION OF BLOCK TO FIRST DECLINE OF CONTRACTION	
			Reflex	Motor nerve
		per cent		
De-afferent preparations....	June 6, 1923	16	2 m. 0 s.	2 m. 10 s.
		14	2 m. 45 s.	3 m. 15 s.
		14	4 m. 15 s.	5 m. 30 s.
		14	1 m. 15 s.	2 m. 15 s.
	June 8, 1923	13	4 m. 40 s.	5 m. 10 s.
		13	3 m. 15 s.	5 m. 0 s.
		13	3 m. 0 s.	8 m. 0 s.
	June 9, 1923	16	7 m. 0 s.	9 m. 0 s.
		16	6 m. 40 s.	8 m. 40 s.
Dorsal roots intact.....	May 28, 1923	12	6 m. 15 s.	9 m. 0 s.
		15	5 m. 20 s.	7 m. 15 s.
		15	3 m. 25 s.	5 m. 30 s.
		16	1 m. 50 s.	2 m. 25 s.
	May 29, 1923	15	45 s.	4 m. 0 s.
		15	55 s.	2 m. 50 s.
	May 31, 1923	15	4 m. 15 s.	12 m. 0 s.
		16	1 m. 10 s.	5 m. 20 s.
	June 7, 1923	13	6 m. 40 s.	6 m. 50 s.
		13	3 m. 30 s.	6 m. 0 s.
		13	3 m. 50 s.	8 m. 30 s.

with that obtaining at a late stage of narcosis when the reflex motor impulses were largely blocked, and relatively much more so than those set up by direct stimulation. In figure 5 A the usual method of recording by connecting the foot with the muscle lever was employed. In figure 5 B the Achilles tendon had been dissected out, severed at its insertion and connected directly with the muscle lever by a thread. This was

Fig. 4. Record showing progress of narcotization of motor nerve in experiment on crossed extension reflex in de-afferent preparation. June 8, 1923. Neuromyal twitches in groups of 3 and 4 recorded in alternation with reflex contractions. Reflex stimuli, 40 break shocks a second. Signal magnet in primary circuit. Time recorded below in seconds. Tests made in pairs; drum stopped after each group of neuromyal twitches to save space, and, in a few cases near end of record, after reflex contractions. First test, 1 min. after block was applied; last test 20½ min. after block applied. Motor nerve stimuli, 30 Z (max. value 14 Z). Reflex stimuli 165 Z to point marked *X*; after that, 200 Z. (Two reflexes showing only small trace of contraction, just before *X*, probably 30 Z units through accidental failure to shift secondary coil.) Alcohol, 13 per cent.

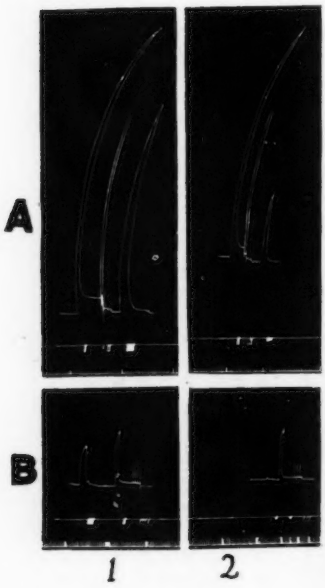

Fig. 5. Selected records showing differential blocking of reflex motor nerve impulses in de-afferent preparation. June 9, 1923. Two separate narcotizations: A, contractions recorded by extension of ankle joint; B, tendon dissected out and connected with muscle lever. In each case, *1*, at time of starting block; *2*, after narcotizing between 15 and 20 minutes. A, first stimulation, tetanizing to motor nerve; second, single shocks to motor nerve; third, reflex stimulation (tetanizing). Stimuli: 60 Z to motor nerve, 120 Z in reflex. B, first, reflex; second, tetanizing to motor nerve; third, single shocks to motor nerve. Stimuli; 94 Z to motor nerve, 165 Z in reflex. In each case drum stopped from 10 to 30 seconds between motor nerve and reflex stimulation.

done in order to eliminate a small amount of irrelevant mechanical disturbance resulting from the contraction of other muscles than those whose motor nerve was being subjected to the narcotic block. These records are fairly typical and seem to leave no doubt that in these cases the motor nerve impulses involved in the reflex were very much more generally blocked than the full-sized impulses set up by direct stimulation of the nerve.

In practically every experiment we noted the course of recovery after the block was removed. The nerve was washed with fresh Ringer solution and records similar to those made during the course of narcotization were taken during its return to normal. The results of these observations differ little from those made during the development of the block, although in some cases the differential recovery of the neuromyal twitch was even more marked than its differential persistence during narcotization. These observations serve chiefly as a control to prove that the reflex had not simply disappeared through some accidental disturbance of the center. An additional control consisted in noting the reflex contraction of other muscles as evidence of the continued efficacy of afferent stimulation. Evidently reflex excitation was setting up impulses in the motor neurones, but they were being blocked by the narcotic, and as the narcotization became more complete they were being blocked in a larger percentage of the fibers than in the case of the full-sized impulses of direct stimulation.

Let us now consider the corresponding results in the preparations that were not de-afferented. In general, these experiments showed a more marked differential effect than those with de-afferent prepara-

tions; i.e., the reflexes were more reduced in proportion to the neuromyal twitch as the block developed. In every instance the reflex disappeared at a time when the neuromyal twitch, though decreasing, was still of considerable magnitude. Figures 6, 7 and 8 show typical examples of this differential blocking.

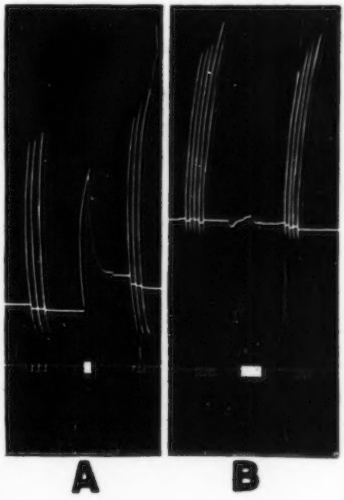

Fig. 6. Records of neuromyal twitch and reflex contraction in preparation with dorsal roots intact, showing effect of partial block on motor nerve. May 28, 1923.

A, 1 minute after beginning of narcotization and before blocking became appreciable; B, 8 minutes after. Drum stopped from 15 to 40 seconds between reflex and motor nerve stimulation. Drop in base line in B during reflex stimulation, due to contraction of other muscles. All stimuli 139 Z. Alcohol 15 per cent.

In describing our method we mentioned the possible confusion due to the blocking of proprioceptive impulses. For the reason there stated, we may in these observations be witnessing not merely a blocking of motor impulses, but in addition an actual diminution of the central activity. Yet we know that the crossed extension reflex does occur in absence of proprioceptive impulses; we may, therefore, assume that the motor neurones were still discharging impulses, though probably in diminished numbers. There is the possibility, which has been suggested in a previous paper (14), that the depression of the crossed extension reflex immediately following the operation of cutting the dorsal roots, may be the inevitable result of interrupting the proprioceptive path at any point; that no matter how the continuity of the afferent fibers from an extensor muscle is interrupted, the crossed reflex involving that muscle is impaired and requires days for its recovery. But in the same paper reasons were given for concluding that the great depression of the crossed extension reflex was due to the disturbance in exposing the spinal cord, and not to the mere cutting of the afferent fibers. If this conclusion is correct, we may assume that in our experiments with the intact dorsal roots, motor nerve impulses continued to traverse the neurones from the reflex center to the block, and the abolition of reflex contraction at a time when many fibers were still able to conduct full-sized impulses to the muscle, was strong corroborative evidence of the differential effect of the block. We are led by these

experiments, as well as by those on the de-afferent preparations, to infer that many of the motor nerve impulses involved in this reflex are unable to pass a block through which full-sized impulses can pass without extinction.

Since the only way we can account for the blocking of an impulse in a fiber in which a full-sized impulse is not blocked is to assume that the blocked impulse is subnormal, we are led to conclude that the motor-

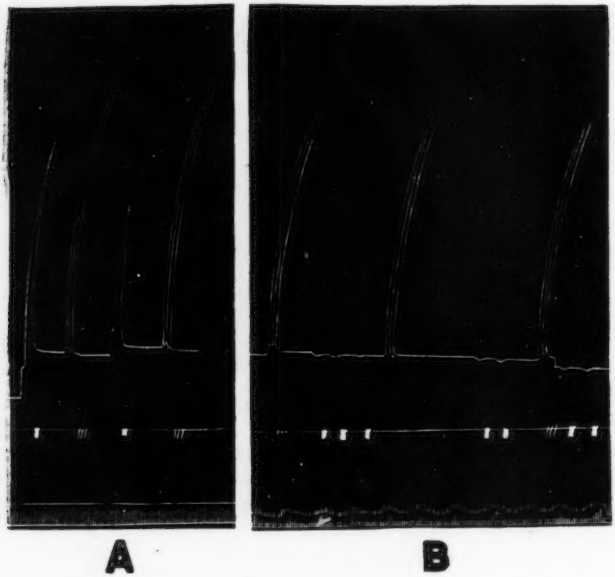

Fig. 7. Records as in figure 6. Dorsal roots intact. May 31, 1923.

A, from 1½ minutes to 5 minutes after application of block with 15 per cent alcohol; beginning of narcosis. Stimuli; motor nerve, 57 Z; reflex, 90 Z.

B, 40 minutes later, from 6 to 8 minutes after application of 16 per cent alcohol. Stimuli; 57 Z in all. (Good reflex responses to stimuli of this strength obtained 5 minutes earlier.) Application of reflex stimulation may be recognized here, as in figures 4 and 6, by solid white blocks in signal magnet record.

neurone discharge in this reflex consists largely in subnormal impulses. And since an impulse, except in a narcotized nerve, is only subnormal when set up in the relative refractory period following a previous impulse, the inference is that many of the impulses in a given motor neurone involved in this reflex follow each other at intervals which our control experiments show to be certainly no more than 3.5σ, and probably no more than 3.0σ.

As just stated, the selective blocking of reflex impulses implies that

some of these are subnormal. The persistence of a small amount of reflex contraction in some experiments until the block became complete suggests that some of the reflex motor impulses may have been full-sized. Yet here we must reckon with the fact, brought out in the control experiments, that all the fibers in the motor nerve do not succumb to the narcotic simultaneously, and indeed that sometimes it is difficult to detect any selective blocking of subnormal impulses. It is impossible to say

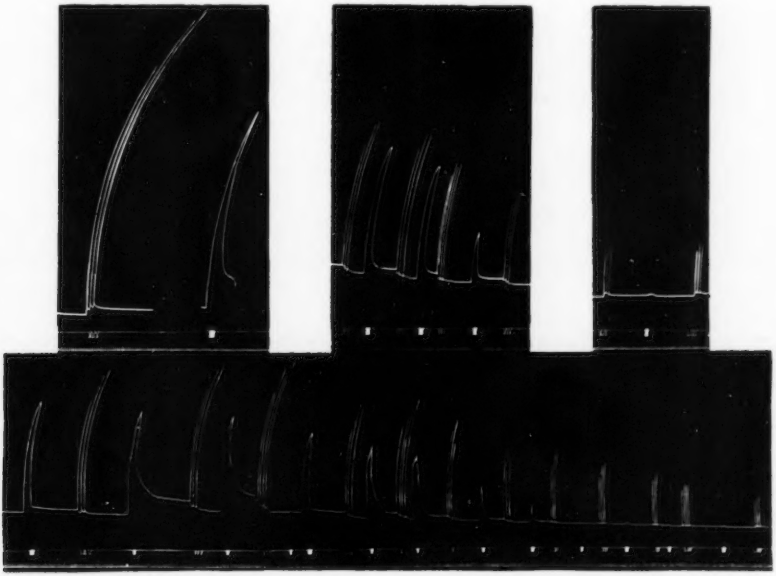

Fig. 8. Records as in figures 6 and 7. Dorsal roots intact. June 7, 1923. Upper row shows selected records of second narcotization in this preparation. First group, before application of block; middle group, from 7 m. 45 s. to 10 m. 50 s. after application; last group, from 13 m. 40 s. to 14 m. 45 s. after application. Lower row shows course of third narcotization from 2 m. 20 s. to 12 m. 30 s. after application of block. Stimuli: motor nerve, 52 Z; reflex, 290 Z.

with certainty to what extent the occasional persistence of reflex contraction till the block is complete is due to the presence of full-sized impulses in the central discharge, and to what extent it is due to unequal narcotization of the component fibers of the nerve. But since in every control experiment there was *some* evidence of selective blocking, we conclude that in those experiments in which the reflex contraction persisted as long as the neuromyal twitch there must have been *some* full-sized impulses in the reflex discharge. Indeed the fact that in nearly

all our experiments the reflex contraction persisted till the neuromyal twitch showed a substantial reduction, inclines us to the view that a certain percentage of full-sized impulses is usually, if not always, involved in this reflex.

Let us turn to the galvanometric records and see if these will throw any further light on the problem. As already mentioned, the electromyogram is more difficult to evaluate as evidence of differential blocking than the mechanical response. In the reflex it is probable that the action currents of various groups of muscle fibers keep getting out of phase with one another, thus neutralizing one another's effect on the recording circuit, whereas in the case of motor nerve stimulation the fibers are forced to respond simultaneously, and thus reinforce each other. The resulting

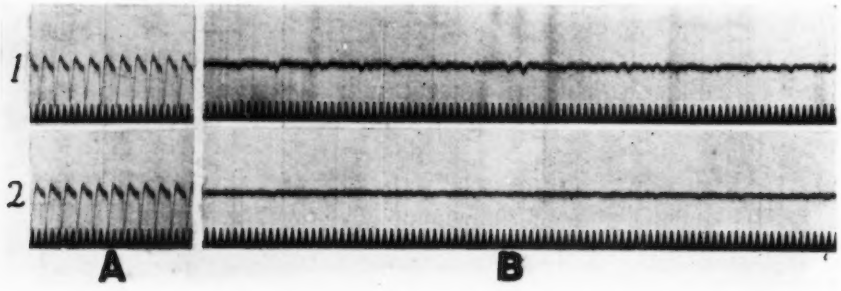

Fig. 9. Electromyograms showing differential blocking of reflex motor nerve impulses in de-afferent preparation. June 9, 1923. A, motor-nerve stimulation; B, reflex stimulation. *1*, 2½ to 3 minutes after application of block. *2*, 21 minutes after application.

Stimuli: 60 Z to motor nerve; 120 Z in reflex; same frequency in both. Hindle galvanometer with 16,800-ohm, gilded quartz string, 1.25 μ diam. Magnification 490. Tension 100 m. per amp. (see Forbes and Ray (44)). Condenser damping with 0.3 mf.

Time shown in each record by shadow of tuning fork, 1 d.v. = 0.01 second.

contrast has been illustrated in a previous paper (14). The irregularity of the reflex electromyogram renders it difficult to find in it any excursions which will be dependable as a quantitative measure of reflex activity. And if the partial block results in any change of action-current rhythm in the muscle fibers, as is not unlikely, such a change will probably vitiate any quantitative comparison based on measurement of excursions. Therefore only if a gross effect is found in the reflex electromyogram while the action current resulting from motor nerve stimulation shows little or no change, can we consider the observation significant.

Another difficulty in the way of interpreting the galvanometer records appeared in some experiments on account of the peculiar shape of the recorded excursion. The action-current record in response to a single

stimulus, applied to the motor nerve, showed three or four phases instead of the common diphasic form which is found when the propagated disturbance travels simultaneously in parallel fibers under a pair of electrodes. We can only interpret this as indicating that in some fibers the motor points lay between the leads, and in consequence the disturbance travelled from these points in both directions at once, and thus complicated the records (1, Ch. III). In these experiments the shape as well as the size of the galvanometric excursions altered as the block developed, and in this way they were rendered unfit for quantitative purposes.

In spite of these difficulties we obtained a few electrical records which reinforced the mechanical evidence in showing a blocking of reflex impulses greater than was the case with those set up by direct stimulation of the motor nerve. This is well illustrated in figure 9, which is taken from a de-afferent preparation. In this case the muscle action currents from motor nerve stimulation were simple diphasic responses and suffered little change when we established a partial block which greatly reduced the apparent size and frequency of the action currents of reflex origin.

It was mentioned above that in the case of a de-afferent preparation the partial block showed a strong selective action on the rate of onset of the reflex contraction; that although the muscle may have ultimately shortened almost as much when the partial block was on as before it was applied, yet the onset of contraction was very much more sluggish. The corresponding change in the electromyogram is illustrated in figure 10. Liddell and Sherrington (19) have shown that the crossed extension reflex is characterized by a process of "recruitment," which means that the reflex begins with activity in only a small percentage of the motor neurones which ultimately take part, and gradually more and more of them become involved. The gradual increase in amplitude of the recorded action currents during the process of recruitment is well shown in figure 10. It is interesting to note that this phenomenon appears so strikingly in the case of a de-afferent preparation. This fact supports the statement of Cooper and Adrian (17) that recruitment is not due in the main to the development of secondary effects, such as tendon reflexes. It is also interesting to note that the time required for the action currents to attain their apparent maximum amplitude is so greatly prolonged by the partial block of the motor nerve. Since in a de-afferent preparation secondary effects due to proprioceptive impulses are eliminated, we may assume that the block has not caused any change in central discharge. We are therefore led to infer that during the early stages of the reflex the proportion of subnormal motor impulses is especially large.

Although, for the most part, the electrical records were less satisfactory

than the mechanical in furnishing quantitative evidence of the selective blocking of reflex impulses, they yielded valuable information in other ways. Cooper and Adrian (17) have published records showing two distinct types of electromyogram in the crossed extension reflex,—one in which the stimulus rhythm appeared in the record in the form of "primary waves" up to frequencies as high as 200 per second, the other in which the action current rhythm was irregular and showed no trace of

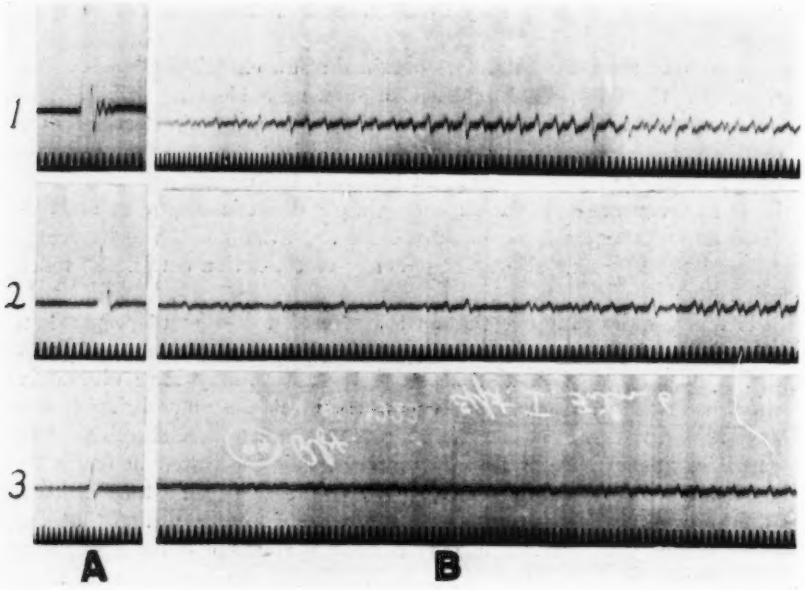

Fig. 10. Electromyograms showing onset of reflex response at three stages in a single narcotization, de-afferent preparation. June 8, 1923. A, single stimuli to motor nerve. *1*, before application of block; *2*, 14 minutes after; *3*, 20 minutes after. B, beginning of reflex at approximately the same times. *1*, 70 seconds after application of block; *2*, 14 minutes after; *3*, 19 minutes after. Galvanometer, string, tension and magnification as in figure 9, but without condenser damping.

the stimulus rhythm. In a previous paper from this laboratory (14) a similar difference in the types of response in this reflex had been noted, but the former type (that showing evidence of the stimulus rhythm) was found far more rarely than in the experience of Cooper and Adrian.

In the present series of experiments we have found both types of response. In one preparation with the dorsal roots intact there was at first no evidence of the stimulus rhythm in the electromyogram. After two

successive narcotizations of the motor nerve the preparation was left undisturbed for three hours. It was then found to be in good condition and the experiment was repeated. The electromyogram taken after this interval revealed clearly the stimulus rhythm. Some change must have occurred in the center which caused its response to change from one to the other of the two types. It is perhaps significant that in the two de-afferent preparations of which we obtained satisfactory electrical records the stimulus rhythm was clearly visible in the electromyogram. This was especially marked in the experiment of June 8, which furnished the records in figure 10. In the experiment of June 9 (fig. 9), the stimulus rhythm is clearly perceptible in the response, but not nearly so marked as in the records of June 8, and no more marked than in the experiment mentioned above, with the afferent roots intact, after the three hours' intermission. As far as these records go, they suggest that the de-afferent preparation is more prone to the type of response in which the stimulus

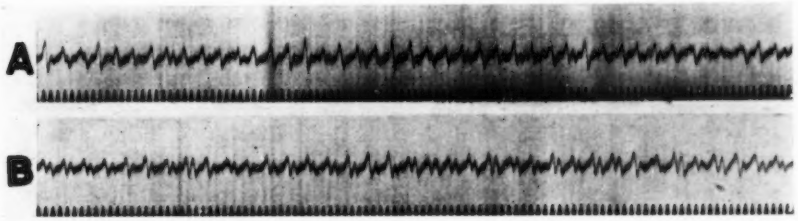

Fig. 11. Electromyograms from the same preparation as in figure 10, showing change in response at height of reflex activity, caused by partial block of motor nerve. A is from the same observation as figure 10, B*1*; B, from the same observation as figure 10 B*2*; in each case after the reflex contraction had reached its maximum.

rhythm appears in the electromyogram than is the intact preparation. This is intelligible if the secondary effect of the proprioceptive impulses serves to render the central activity more sustained. If more of the motor neurones were constantly occupied in consequence of this secondary effect there would be less likelihood of a large group of them being free to respond immediately and simultaneously upon the arrival of each volley of afferent impulses. Still, our results on this point are too meager to justify any such conclusion; we should consider it no more than a mere suggestion.

In the experiment of June 8—that in which the stimulus rhythm was most clearly revealed in the action currents—we found an interesting change in the electromyogram as the block progressed. This change is illustrated in figure 11. The upper record, A, shows the electromyogram immediately after the alcohol was applied and before it had produced any appreciable change in the form of response. The lower record, B,

was made 14 minutes after the block was applied and 9 minutes before the muscle ceased to contract in response to the reflex stimuli. It will be noticed that in the first record each primary wave is followed by a period in which the galvanometric excursions are very small; practically the only large excursions are the "primary waves" corresponding regularly with the afferent stimuli. In the second record, on the other hand, while the primary waves are still clearly recognizable as the largest excursions, more than half of these are followed regularly, after an interval of approximately 10σ, by substantial secondary excursions, in many cases almost as large as the primary. This effect was also seen in the next reflex record taken twenty minutes after the application of the block; moreover, after the block was removed the electromyogram became again essentially like that shown in figure 11, A.

This change in the form of record, if properly interpreted, may furnish valuable information as to the rhythm of motor nerve impulses involved in the reflex. In previous papers (28), (40) reasons have been given for believing that in a reflex characterized by after-discharge, as is the crossed extension reflex, nerve impulses approach the motor neurones, not only through the most direct available path from the afferent neurones, but also through more indirect paths which are to be found in the complex structure of the spinal cord, designated for convenience "delay paths." In a preparation such as that under consideration, in which the stimulus rhythm is conspicuously revealed by primary waves in the reflex electromyogram, it is reasonable to suppose that a large percentage of the motor neurones in the center are promptly excited by impulses arriving almost simultaneously from the afferent nerve. The small secondary waves may be the result of activity in motor neurones which failed to participate in the production of the primary waves, or they may be the result of repetitive discharge from the same neurones which produced the primary waves, evoking a second response in the muscle fibers. The question arises, why when the partial block is applied to the motor nerve, should the more irregular series of very small secondary waves be replaced in most cases by a single comparatively large and well-defined wave occurring with considerable regularity at approximately 10σ after the primary wave?

Suppose each afferent volley arriving at the center finds the motor neurones for the most part inactive, and, therefore, ready to respond with an almost synchronous volley of full-sized motor impulses. This will explain the persistence of the primary wave in almost undiminished size even after the block has begun to modify the electromyogram. Suppose then that each motor neurone, after discharging a full-sized impulse, is caused by impulses reaching it through the "delay paths" to discharge a rapid series of subnormal impulses, subnormal because they occur so

rapidly. It has been shown possible for the second of a series of nerve impulses to occur so early in the relative refractory phase following the first that it fails to evoke a second response in the muscle (21). There is no reason to suppose that all the nerve fibers or all the muscle fibers do precisely the same thing in effecting a reflex response; in fact the irregularity of the electromyogram gives every reason to suppose that they do not. We might find, for example, that some motor neurones discharge only a single full-sized impulse at the time of each primary wave. Others may discharge only two, and of these some might evoke two responses in the innervated muscle fibers, and some only one. Others may discharge a series of three or four impulses, and of these some may discharge them at such intervals that the muscle could respond to every one, while some might discharge them with so high a frequency that only the first and third would excite the muscle. Still other neurones might be occupied and therefore refractory at the time of the primary wave and would therefore fail to respond. Others in the relative refractory phase would be unable to discharge full-sized impulses at this time; they might then respond to the afferent volley with subnormal impulses, and such of the innervated muscle fibers as were not too refractory would then respond. Such an assortment of nerve impulses and resulting muscle responses would furnish just such a picture as we find in the electromyogram of figure 11, A, the synchronous muscle responses in a large number of fibers causing the primary waves, and the early second responses of scattering groups of fibers, after somewhat varying intervals, giving rise to the small secondary waves.

To explain the effect of the block in changing the picture to that shown in figure 11, B, we must find some way to account for its enabling some of the muscle fibers, previously unable to do so, to respond at about 10σ after the primary wave, or some fibers whose responses were previously subnormal to give a larger response at this time than was possible before the block was applied.

A simple interpretation of this change would be as follows: Supposing each group of motor nerve impulses to begin with one of normal size which gives rise to the primary wave in the muscle record, followed in each neurone by an after-discharge consisting at first of impulses of high frequency, and becoming gradually less and less frequent, there would be a corresponding gradual change in the size of the impulses; the first part of the after-discharge would consist of subnormal impulses, and as they became less frequent they would become larger. The fact that with a certain degree of narcosis, a well-defined muscle response appears in the record fairly regularly at about 10σ after the primary wave, would simply mean that at about 10σ after the main volley from the center the impulses begin to be large enough, in most of the neurones, to pass through the

block. The first successful impulse in the after-discharge might be the third, fourth or fifth of the whole series, but in most cases it would occur in the neighborhood of 10σ after the main volley, and thus would initiate a substantial group of muscle responses at about this time. This seems to us the simplest and most reasonable interpretation of this change in the electromyogram in consequence of the block. Of course, the transition in the after-discharge from the frequent, subnormal impulses, which are blocked, to the less frequent and therefore larger ones, which pass, might be gradual or abrupt. The experimental evidence merely seems to show an increase in the sum total of muscle response at about 10σ after the primary wave, and this is most easily explained by assuming that the nerve impulses following the primary wave are subnormal up to about 10σ, and after that, are full-sized, or at least large enough to pass the block. We should emphasize that the electromyogram in figure 11, B does not by itself suffice to indicate the presence of subnormal nerve impulses at an earlier stage in the after-discharge than 10σ after the primary wave. But taken in connection with figure 11, A, it is significant; it shows an increased amount of response at 10σ, which appears to denote the stopping of some intervening activity which had previously served to cut down the activity at this time,—presumably subnormal nerve impulses evoking early muscle responses.

We found this effect in only one experiment, and cannot, therefore, say whether it would prove to be a common occurrence. But in this preparation it occurred with a regularity which renders the observation significant.

The main facts revealed by our observations are that in the crossed extension reflex a substantial percentage of the motor nerve impulses are subnormal and therefore follow their predecessors after intervals of less than about 3σ; but that usually some are full-sized or nearly so, and therefore occur more than 2.5σ and probably more than 3σ after their predecessors. The conclusion, therefore, is that the rhythm of discharge in any given motor neurone is not uniform, but highly irregular. The impulses are probably discharged in groups separated by periods of comparative inactivity.

Discussion. In resting tissues when two stimuli are applied to a motor nerve, only in the earliest stage of the relative refractory phase can a second impulse be evoked which fails to evoke a second response in the muscle. But since the muscle, with its longer refractory phase, responds after an added delay, it becomes easier to time the third impulse so that the muscle will fail to respond to it. Thus as the series of rapid nerve impulses is continued, the muscle fibers tend increasingly to fail to respond to them. In a prolonged series of nerve impulses too rapid for the muscle to keep pace, the lagging muscle fibers must ultimately

fall so far behind as to skip one nerve impulse in the series, but will respond to the next (see 12, pp. 592–602); thus even a perfectly regular series of nerve impulses may set up an irregular series of muscle responses with a slight break in rhythm whenever a nerve impulse is skipped. This is one factor making for irregularity in the electromyogram, which should be borne in mind in the ensuing discussion. We might find a motor nerve rhythm of 600 resulting in this way in a muscle rhythm of between 200 and 400 per second, but we could not expect so large a difference as is suggested by the analysis of Forbes and Rappleye.

But the results in the present paper show that it is useless to talk about a *regular* rhythm of motor nerve discharge of *any* frequency in the crossed extension reflex, for one of the most salient facts which appears to emerge from our evidence is the great irregularity of the rhythm. Since some of the impulses are full-sized and therefore occur probably at least 3σ after their predecessors, while others are subnormal and therefore must occur less than 3σ after their predecessors and perhaps after intervals as small as 1.7σ, we must look on the discharge as having no regular rhythm.

Cooper and Adrian (16) in their study of the flexion reflex concluded that the spinal cord could not discharge impulses at a higher frequency than about 300 per second. They say "The smallest interval between two successive impulses leaving the cord lies between .003 and .004 second." This conclusion appears in sharp contrast with our results obtained with the crossed extension reflex, showing that some impulses follow their predecessors by less than 3σ. The question arises whether this difference is due to a difference between the crossed extension reflex and the flexion reflex. This may be so, yet it does not seem to us a necessary inference, for as we have already intimated in the introduction, the evidence of Cooper and Adrian does not seem to us convincing proof of their conclusions. Let us now examine this question. Their principal arguments are that as they increased the frequency of the electrical stimuli applied to the afferent nerve to evoke the flexion reflex the resulting electromyogram continued to show the stimulus rhythm until this was raised to a frequency of about 300 per second, and that this limit was not due to the muscle's inability to respond with a higher frequency, for when the stimuli were applied to the motor nerve, the muscle was able to follow up to 400 per second; that as they cooled the spinal cord, keeping the muscle at normal body temperature, the upper limit of frequency, at which the electromyogram could show clearly the stimulus rhythm, was progressively lowered and showed a continuous correlation with the temperature as far as the cooling was carried (i.e., to 28°C.). From this they inferred that the actual frequency of discharge from the individual motor neurones was that which appeared in the electromyogram, and

depended on the refractory phase of some part of the mechanism in the spinal cord. This assumes that the apparent frequency in the electromyogram, taken from the muscle as a whole, faithfully represents the real frequency of response of the individual constituent fibers. But we must constantly bear in mind the fact that the electromyogram is a composite picture, for the muscle consists of thousands of independent groups of fibers. Accordingly we must treat evidence obtained from the muscle as a whole on a statistical, rather than an individual basis. Unless it can be shown that each excursion in the electromyogram represents

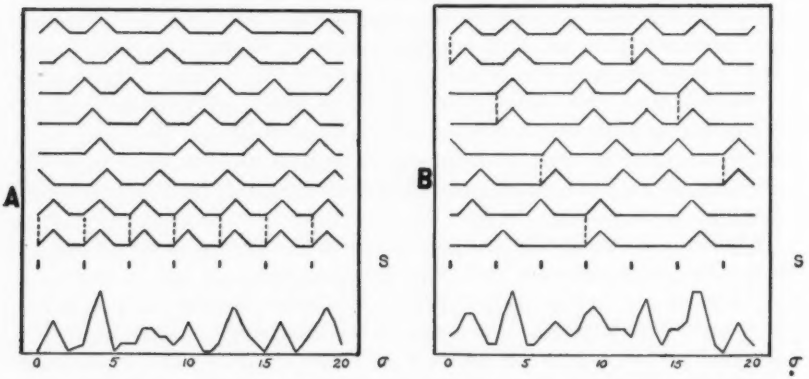

Fig. 12. Two hypothetical cases of composite pictures of electrical response of muscle consisting of 8 groups of fibers, with stimuli delivered at 333 per second. In each, the times at which unoccupied fibers would begin their response to each stimulus, are shown by the marks in the row designated *S*. Groups responding to each stimulus are connected with vertical dotted lines. The apparent response of the whole muscle, obtained by adding ordinates, shown below. Time in σ at the bottom of each record. In each case 6 groups are occupied in irregular series of responses, and 2 groups respond to each stimulus. In A the same groups respond every time; in B the groups respond in rotation by pairs. In A the frequency of response in the individual groups in haphazard activity varies from 207 to 286 per second, average 242. In B the individual frequency varies from 154 to 275 per second, average 235

activity in all or practically all the constituent fibers of the muscle, there is no proof that the same fibers participate in any two successive responses. When the stimulus rhythm appears in the electromyogram all it really shows is that following each stimulus *some* fibers are able to give a response, but they may not be the same fibers as those which gave the previous response. It is easy to show by a graphic plotting of the hypothetical responses of a series of separate units that even a small minority of fibers keeping step with the stimulus rhythm, while all the rest of the fibers are responding in a haphazard, irregular way, would make the stimulus rhythm appear very clearly in the resulting record. This is shown in

figure 12, in which we have plotted the results of various groupings of individual muscle responses in two hypothetical cases; one in which a small minority of the fibers are keeping step with the stimulus rhythm while the majority respond in a wholly irregular way; the other in which each stimulus excites the same percentage of fibers to synchronous response, but each time a different group. For simplicity in plotting, the responses are shown as monophasic and all full-sized, and the curves have been made rectilinear, but we feel that it serves to illustrate the principle.

Thus the rhythm corresponding with that of the stimuli, which appears in the records of Cooper and Adrian, may have been due to the responses of only a small minority of the fibers in the muscle; and indeed as far as one can judge from their electromyograms, it is possible that none of the individual fibers were following the stimulus rhythm.

Let us now analyze the possibilities in the matter of motor-neurone discharge and see if an explanation of the facts observed by Cooper and Adrian can be found which will be more concordant with our observations than the interpretation which they offer. Let us first consider the experiments at normal body temperature. A thorough exploration of the possibilities requires due allowance for the branching of afferent fibers and the resulting complexity of their central connections. Anatomically this branching is known to be extensive. Evidence of its physiological importance has been emphasized in previous papers (40, p. 378), (12, p. 610). As Adrian says, "extensive central connections must exist so that the impulses arriving from the different fibres of the sensory root are pooled, as it were, in a common network and subjected to further synthesis and rearrangement before the result is finally handed on to the neurones of the motor centre." (41, p. 414). This branching of afferent fibers into a large number of central conduction paths has been made the basis of a simple interpretation of after-discharge (40). In discussing the electromyogram in figure 11, A (p. 47), we have already sketched some of the possible groupings of motor nerve impulses which may well result in this way from the afferent volleys. We pictured the motor neurones as being excited first by impulses coming over the most direct paths from the afferent fibers, and then by impulses traversing "delay paths." There need not be any sharp division between the two types of path; there may be all intermediate gradations between the most direct and the most roundabout.

The decerebrate crossed extension reflex shows so much more after-discharge than the spinal flexion reflex that it might seem impossible to apply any argument based on observations of one reflex to the problem of motor-neurone discharge in the other. Yet even in the spinal flexion reflex, on which Cooper and Adrian based their arguments, there is usually

some after-discharge, as revealed by their electromyograms (16, fig. 7), (21) and as was strongly suggested by evidence of a different sort from this laboratory (22). Therefore we believe that the difference is one of degree, and that the principle of branching and converging paths in its bearing on after-discharge, applies to all cases.

Of course after-discharge may depend on some property of the synapse whereby it is thrown into a state of sustained activity altogether different in kind from the peripheral nerve impulse with its transient character and correlated refractory phase (cf. 41). But it seems to us simpler to assume that the impulse is of the same nature in all parts of the conducting fibers (40). In either case the problem becomes much the same when we consider the discharge of impulses in the motor neurones. Whether the central activity consists of some sustained disturbance of a different kind at the synapse or of a rapid bombardment of the motor neurone by impulses converging upon it from many paths, the resulting discharge must appear in the motor nerve fibers as individual impulses, limited as to frequency by the well-known refractory period of these fibers.

Now it is natural to suppose that following the arrival of a volley of afferent impulses in the central network there will at first be a rapid convergence of impulses at the motor neurone which will tend to evoke motor impulses of high frequency; also that, failing a fresh afferent volley, the central impulses will arrive less and less frequently till the after-discharge dies away altogether, and as the frequency of converging central impulses becomes less than the maximum possible frequency of response in the motor neurones, the motor impulses become less and less frequent. We might imagine the same result in the case of excitation by a sustained disturbance of a different sort at the synapse, but in this case it is hard to see how the interval between successive motor impulses thus evoked could exceed the time of complete recovery of the motor neurone from its refractory phase; whereas if the central activity consists of individual nerve impulses, the interval might increase to any extent, limited only by the conduction time of the longest path. The evidence contained in figure 11 in the present paper, showing the change in the electromyogram, caused by a partial block, supports the view that in the crossed extension reflex the motor nerve impulses may be so frequent as to be subnormal till about 10σ after the initial volley, and then lapse to a frequency which permits full recovery between impulses. In the case of the flexion reflex, in which there is less after-discharge, we might expect a similar condition, but with a more rapid cessation of activity after each volley.

Let us apply these considerations to the experiment of Cooper and Adrian in which the stimulus frequency was raised till it disappeared from the electromyogram. Even in the records of low frequency at which this rhythm is still visible, the excursions are so small and irregular as to show

clearly that by no means every muscle fiber took part in each successive response. We may suppose that some motor neurones were being constantly thrown into a haphazard, irregular activity by the central after-discharge. A majority, even, may have been so occupied, but each afferent volley found enough motor neurones unoccupied to evoke in them a prompt, synchronous response which could excite enough muscle fibers to produce a perceptible excursion in the record. As the stimulus frequency was increased we may suppose that more and more motor neurones became involved in the high-frequency after-discharge, which rendered them refractory at the moment when the first impulses of each afferent volley arrived; thus a smaller and smaller minority would be free to discharge the synchronous motor volley required for a visible excursion. Finally, when practically all the motor neurones were involved in an almost steady bombardment of converging impulses in the overlapping after-discharges, the stimulus rhythm disappeared from the electromyogram, leaving only the irregular oscillations of a haphazard grouping of muscle responses.

What is the significance of the critical frequency above which the stimulus rhythm disappears from the response? Since below this critical frequency the electromyogram, though revealing the stimulus rhythm, gave no certain evidence that any two successive excursions were caused by the same group of muscle fibers, the records tell us nothing of the real frequency of response in these fibers. The critical frequency, then, would be that at which the after-discharge from each volley maintains such a general state of haphazard activity that no motor neurones are left free to respond to the next volley with synchronous, full-sized impulses, or at least not enough to impress their rhythm on the resulting record.

When the stimulus rhythm has disappeared, and the apparent frequency in the record has become wholly irregular, the question of the frequency of response in the individual muscle fiber, is, if possible, even more uncertain. In the human electromyograms of Piper, made with powerful voluntary contractions, there was frequently a dominant rhythm of large diphasic excursions, each of which resembled closely that of the response to a single induction shock applied to the motor nerve (1, p. 97). In that case it was fair to assume that most of the fibers participated in the major excursions of the electromyogram, and that therefore the observed rhythm revealed a true frequency of muscle response. But in the records of Cooper and Adrian the picture is quite different. When the stimulus frequency was too high to appear in the record the observed rhythm became quite irregular, and the individual excursions were all of them small compared with a maximal response of the whole muscle; the condition more nearly resembled that shown in figure 13 of a previous paper (14), in which the small irregular excursions in a vigorous crossed

extension reflex were contrasted with the maximal action current of the same muscle. In this case we are not justified in supposing that any excursion is caused by a majority of the fibers, or in interpreting the record as showing anything but a haphazard grouping of individual responses. Here the apparent frequency shown by counting every visible excursion in the record seems to depend chiefly on the mass of the string, as shown in a previous paper (14, p. 157). It should be clearly understood that we do not refer to any *natural period of vibration* in the string. In all these records, both those of Cooper and Adrian and the others cited, the string was slacked to the limit of periodicity, so that no appreciable oscillations could have been introduced into the record by the string vibrating in its own period. What we mean is that if a fluctuation in electric current is small enough and brief enough, the inertia of the string will mask it, no visible notch or excursion appearing in the record; and the *mass* of the string, irrespective of tension, sets the limit to its analyzing power. Of course, a string can be forced to record currents of very brief duration if they are strong enough, but the briefer the current the stronger it must be to cause a visible excursion.

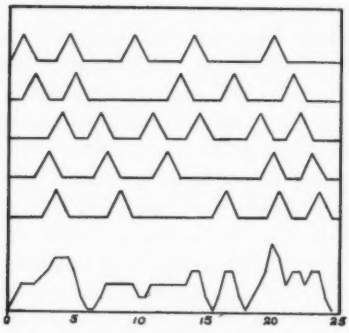

Fig. 13. Hypothetical record of response in muscle of five groups of fibers responding irregularly and out of phase with one another. See text. Time below in σ.

In figure 13 we have shown a hypothetical case of 5 groups of fibers, all responding with irregular frequency and all out of phase with each other, plotted in the same way as figure 12. The average frequency of response of the individual groups is 207 per second. The frequency of the notches appearing in the record would be 167, 290 or 375 per second, according to the smallness of the fluctuations which the string could reveal.

Cooper and Adrian mention that when the sensory nerve is stimulated so rapidly that the electromyogram becomes irregular its apparent frequency is usually lower than the critical frequency. The significance of this in terms of the interpretation we are developing, is not clear. Two factors might enter into its causation: The prevalence of very high frequency of impulses in the motor nerve would cause increasing numbers of muscle fibers to respond only to alternate impulses, instead of to every impulse in a series. This might result in the slowing of the actual muscle rhythm in many fibers and might cause an apparent slowing of the frequency revealed by the composite picture. The other possible factor is

the merging into a single excursion of the responses of two or more groups of fibers, not quite synchronous, as their electric responses increasingly overlap. Since the string can register a higher frequency than appears, there is some doubt whether this latter factor could be expected in the long run to cause an actual decrease in the apparent frequency. But we contend that the frequency of individual muscle-fiber responses will influence the electromyogram in a wholly unpredictable way, and it would be rash to draw any inference as to this frequency from these irregular records.

The other experiment of Cooper and Adrian, in which they showed that cooling the spinal cord, while the muscle was kept at normal body temperature, lowered the critical frequency above which the stimulus rhythm ceased to appear in the record, is very significant; yet a statistical treatment of the results in this case also shows that their inference is not a necessary conclusion. Their records show that cooling the cord lowers the frequency which can be made to appear in the electromyogram. This means, presumably, that at any given frequency of stimulation, cooling must decrease the percentage of motor neurones which are free to respond synchronously on the arrival of each afferent volley. Now their evidence in figure 7 on page 73 (16) shows quite clearly a decrease in the tendency to after-discharge when the cord is cooled. If there were no other effect, the motor neurones would be all the more ready to respond each time an afferent volley arrived in the center, and cooling would then make the stimulus rhythm at a given frequency stand out more clearly in the electromyogram. To explain the opposite result we must conclude that there is some other change in the center. The change might be such as Cooper and Adrian have suggested; namely, some portion of the central conducting path may have a refractory period long enough to limit the frequency with which impulses can traverse it to the critical value above which the electromyogram becomes wholly irregular. Or it might result simply from a slowing of conduction in the various paths leading from the afferent fibers to the motor neurones. Suppose that the afferent fibers are all connected with the motor neurones by direct paths whose conduction times at normal body temperature differ from each other only enough to account for the dispersion in time of the first volley of motor impulses revealed in the experiments of Forbes and Gregg (27, pp. 148–154). We may also suppose that the branched arrangement of central connections provides other paths of longer conduction times. If cooling the cord caused the same relative prolongation of conduction time in all these paths, this would result in throwing the responses of the motor neurones far enough out of phase with each other to break up the previously visible rhythm in the electromyogram.

Even supposing we accept the conclusion of Cooper and Adrian, that

some portion of each central conducting path from afferent fiber to motor neurone has a refractory period which limits the frequency of impulses that can traverse it to about 300 a second at normal body temperature, and to progressively slower frequencies as the cord is cooled, it is easy to see that this does not necessarily lead to their further conclusion—that "the smallest interval between successive impulses leaving the cord lies between .004 and .003 second" (16, p. 77). For as Adrian has pointed out (41, p. 414), the branched arrangement of the central network provides for a "pooling" of impulses which would probably result in a frequency of discharge from the motor neurones quite different from the limiting frequency imposed on the individual central pathways by this assumed refractory period. Even if impulses could not traverse the particular central path more frequently than 300 a second, impulses converging upon a given motor neurone through two or more such paths in alternation or rotation, might easily cause it to discharge impulses with any frequency up to the limit (600 or so) which the refractory period of the motor fibers imposes.

Cooper and Adrian used a third line of evidence in support of their conclusion (16, p. 75). They evoked the flexion reflex by sustained mechanical stimulation applied to the foot, instead of by electrical stimuli applied to the afferent nerve. They varied the temperature of the muscle without altering the temperature of the cord, and recorded electromyograms at the various muscle temperatures. Cooling the muscle produced no change in the frequency of the excursions they were able to count in the electromyogram. Yet in a control experiment in which the muscle was excited through its motor nerve by stimuli between 640 and 800 a second, and its temperature was varied through the same range as in the reflex experiment, the electromyogram, though irregular and failing to show the stimulus rhythm, nevertheless did show a decrease in the frequency of visible excursions with cooling.

This evidence strongly suggests that the frequency of the excursions depends upon the temperature of the cord and not upon that of the muscle, and in this way tends to support the conclusions they drew from the other lines of experiment. But there is another possible interpretation which has not been excluded. In this case, a sustained mechanical stimulus being used, there is no tendency whatever to start the reflex effect through synchronous afferent volleys, as is artificially done in the case of electrical stimulation. Synchronous afferent volleys tend to impose their rhythm upon the central discharge, as has already been explained, even though no single motor neurone follows the rhythm in question; but when the afferent impulses are set up in a purely haphazard way—there being no stimulus rhythm—there is no tendency to create such a rhythm in the central discharge. The responses of the motor neurones and of the muscle

fibers will tend to get in and out of phase with each other in an equally haphazard way. Under these circumstances it is altogether likely that the frequency found by counting every visible excursion in the electromyogram will depend mainly upon the mass of the string, in the manner already discussed. If this is so, it is quite possible that although the motor nerve discharge is of higher frequency than the muscle can follow, yet cooling the muscle would cause no change in the apparent frequency determined in this way.

In the control experiment, on the other hand, the stimuli applied to the motor nerve, although too frequent to be followed by a regular response in the muscle, nevertheless create a tendency for the responses of groups of muscle fibers to be synchronized at times determined by the times of stimulation. Even if the resulting record is irregular, the apparent frequency will tend to have some relation to the stimulus rhythm. The intervals between the successive responses of individual muscle fibers will tend to be multiples of the interval between stimuli, although perhaps not always the same multiple; and as the refractory phase of the muscle fibers is prolonged by cooling, the interval between successive responses will tend to become a larger multiple than before. It is difficult or impossible to predict the resulting composite picture from such responses when the stimulus frequency is so high that the electromyogram has become irregular, but it seems to us possible that in this case cooling the muscle might decrease the frequency of excursions, although no such effect could be seen in the case of mechanical stimulation, which provided no tendency whatever to synchronous volleys at any point in the reflex arc. Thus, while this evidence tends to make the frequency appearing in the electromyogram dependent on the refractory phase of some part of the cord, it is possible that this piece of evidence, as well as the others discussed above, may be capable of a different interpretation.

We conclude that although the evidence presented in this paper of Cooper and Adrian suggests the possibility that some part of the conducting mechanism of the spinal cord has a refractory period which limits the frequency of impulses traversing it to about 300 a second at normal body temperature, nevertheless their results can also be explained on the ground of diversity of conduction time in the branching and converging paths. Clearly it is not disproved that the motor neurones may, as a result of converging paths, be caused to discharge impulses with a frequency as high as 600 a second.

We do not claim to have *proved* by our reasoning that in the flexion reflex the motor-neurone discharge is of the rapid and confused character that we have just depicted. It is possible that the conclusion which Cooper and Adrian drew from their experiments was correct,—that the frequencies appearing in their electromyograms represented the actual

frequency of discharge of the individual motor neurones. What we contend is that this conclusion does not necessarily follow from their observations. All of their experimental results seem to us compatible with the picture of central discharge we have drawn, involving irregular discharge of impulses, often of higher frequency than the muscle fibers can follow.

This brings us back to the question whether the presence of impulses of high frequency in the motor nerve, shown by our experiments to be a dominant character of the crossed extension reflex, is peculiar to that type of reaction, or whether it is characteristic of sustained reflexes in general, including the flexion reflex when evoked by sufficiently frequent afferent stimuli. Since the flexion reflex—especially in the spinal animal—is marked by a much briefer after-discharge than is found in the extensor reflex, we may suppose that volleys of full-sized motor impulses are apt to occur more frequently and to play a larger part in the former than in the latter. But when the afferent stimuli are so frequent that the after-discharges presumably overlap, and the stimulus rhythm disappears from the electromyogram, we see no valid reason for supposing that the motor-neurone discharge in the flexion reflex differs much from that in the extensor reflex. Since we find no real proof that the motor neurones discharge their impulses with a frequency so low that the muscle can follow it, we are inclined to suppose that in sustained flexion, as in the crossed extension reflex, groups of impulses often occur with so high a frequency as to render them subnormal,—a frequency too high to be followed by the muscle fibers.

We conclude, then, that in the crossed extension reflex the frequency of motor-neurone discharge is irregular, usually some groups of impulses in a given neurone having a frequency between 330 and 600 per second for a time; but such groups are probably separated by occasional lulls. We further conclude that this condition is probably general for sustained reflexes, including the flexion reflex when evoked by stimuli of high enough frequency to produce a complete merging of successive central effects.

In their experiments on the frog, Cooper and Adrian (7) made a somewhat stronger case than they did in their mammalian experiments, for their electromyograms showed larger and more regular excursions, suggesting the synchronous activity of a larger proportion of the muscle fibers and a greater probability of a true muscle rhythm, and they obtained definite and significant rhythms with both electrical and mechanical stimuli, in each case with the temperature of both cord and muscle independently raised and lowered. In particular they obtained a significant decrease in frequency from *mechanical* reflex stimulation on cooling the cord, a procedure which was lacking in their mammalian work, and

the lack of which lays their argument open to the criticism we have given on page 51. We are not certain that a statistical treatment of their results on frogs could not be found which would open the way to a different interpretation, but their observations certainly appear to be most easily interpreted in accordance with their conclusions.

It seems to us a likely possibility that the reason for their conclusions concerning the frog appearing correct and those in the case of the mammal, incorrect, is that these two classes of animals are differently constituted. We know that the reflex centers in the mammalian cord are far more highly organized than in the amphibian cord; and a feature of this organization may well be a much greater extent of branching of central connections and of the resulting tendency to after-discharge. This view receives some support from the observations of Tiedermann (43), (cf. 32, p. 96) in which he produced in the frog a change from reflex excitation to inhibition by increasing the stimulus frequency, as in the case of the Wedensky effect. This cannot be so simply done in the mammalian preparation, a fact which suggests the more complete breaking up of the stimulus rhythm in consequence of central branching.

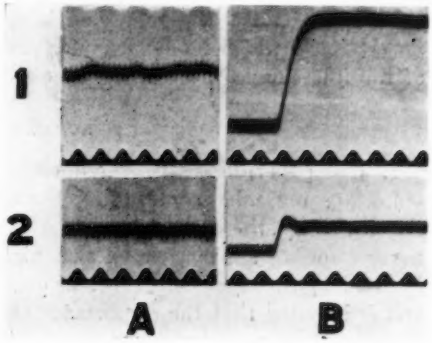

Fig. 14. Demonstration of the ability of a slack string to reveal currents of brief duration as well as a tight string. A, direct current interrupted by rotary interrupter approximately 400 times a second (50 per cent closure). B, calibration curve showing excursion on prolonged closure of the same current. 0.004 volt through 17,000-ohm string in series with 20,000 ohms. Diameter of string, 2.75 μ, magnification 490. *1*, tension 70 m. per amp. *2*, 280 m. per amp. Tuning fork shadow 1 d.v. = 0.01 second.

Athanasiu (42) has recently raised other objections to the arguments of Cooper and Adrian in connection with their earlier experiments on the frog (7). But his arguments are based on assumptions which have already been refuted (14, pp. 165–169), and need not be considered here. One point is worth mentioning, as it is open to a very simple experimental refutation. Athanasiu states that a slack string cannot follow the rhythm of rapid oscillations. Now it is quite true that a slack string cannot give as faithful a record of the time relations of currents of brief duration as a tight string, but it can reveal their presence just as clearly, as we have shown in figure 14. This shows records of intermittent currents made first with a tight string and then with the same

string four times as slack. We mention this point since it is of some importance in the interpretation of electromyograms and other records involving the detection of small currents of brief duration.

We find no adequate reason to discard the view of Forbes and Rappleye, that in voluntary contraction the nerve-impulse frequency is too high for the muscle to follow. We incline to look on the central discharge in this case as probably similar to that in the crossed extension reflex. But in the case of the human forearm flexors, which have usually been made the subject of the experiments, the presence of a dominant, major rhythm of less than 100 per second, and the lack of direct observations on the refractory phase of these muscles, introduce quantitative differences and uncertainties which render it premature to carry over to this case many of the inferences drawn from reflexes in the cat.

SUMMARY

1. This paper deals with the question whether in sustained reflex and voluntary muscular contractions the excursions in the electromyogram show directly the frequency of motor nerve impulses, or whether the nerve impulses are discharged at a higher frequency not directly shown by the major excursions. Previous evidence on this question is reviewed, and it is noted that the significance of all this evidence depends on the interpretation of the electromyogram itself.

2. In an attempt to obtain more direct evidence we have employed Lucas' method of establishing a partial block in the motor nerve by means of dilute alcohol. With this procedure we have examined the crossed extension reflex in the decerebrate cat, this reflex serving as a good example of sustained muscular contraction of central origin.

3. The principle of the alcohol block method is as follows: A partial block will stop subnormal, but not full-sized impulses; according to the all-or-none law, the size of the impulse depends not on the strength of stimulus but on the degree of recovery from a previous response, i.e., a subnormal impulse occurs only in the relative refractory phase and therefore at a brief interval after its predecessor; the stopping of nerve impulses by a partial block therefore proves them to be of high frequency in the individual fiber.

4. Control experiments showed that, as we used the block, it stopped impulses following their predecessors at intervals between 1.7σ and about 3σ.

5. Some of our experiments were made on de-afferent preparations, to avoid the complication of proprioceptive impulses; some were on animals with the dorsal roots intact. We recorded the mechanical contractions of the gastrocnemius muscle in all experiments, and in most of them we also recorded its electromyogram.

6. Both groups of experiments showed that in this reflex a considerable percentage of the motor nerve impulses, and probably a majority of them, were subnormal, and therefore followed their predecessors by less than 3σ. The persistence of a slight reflex contraction in some preparations almost or quite to the time the block became complete, seemed to show that a few of the motor nerve impulses involved were full-sized, and thus to reveal an occasional lull between groups of impulses of high frequency.

7. One preparation showed consistently and strikingly a change in the electromyogram, as the block developed, which seemed to signify that after each afferent volley the motor neurones were steadily occupied in a high-frequency after-discharge for about 10σ, at which time the motor nerve impulses became full-sized, indicating a decrease in their frequency.

8. The results are compared with those of Cooper and Adrian on the spinal flexion reflex, which led them to the conclusion that in this reflex the motor neurones never discharge with a higher frequency than 320 per second. It is shown that this conclusion is based on the interpretation of the electromyogram as recording the true frequency of response of representative muscle fibers, and that this interpretation is not necessarily valid. Analysis of their evidence, treating the electromyogram as a composite picture of the activity of many independent fibers, shows another interpretation compatible with the view that in the flexion reflex, when sustained, as well as in the crossed extension reflex, the frequency of motor nerve impulses may often be more than 330 per second.

9. The evidence of irregularity of discharge is such that no reflex should be supposed to involve a perfectly regular series of impulses at any constant frequency.

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EXPERIMENTAL CONTRIBUTION TO THE STUDY OF THE RELATION BETWEEN NIGHT BLINDNESS AND MALNUTRITION

Influence of Deficiency of Fat-Soluble A-Vitamin in the Diet on the Visual Purple in the Eyes of Rats

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I. Historical introduction. On the basis of clinical observation many investigators have suggested a relation between xerosis conjunctivae and certain kinds of night blindness in human beings. These two ailments are often found jointly in the same patients and under the same circumstances. Their clinical relation was recognized by Bitot in 1863 (3) who first described xerosis conjunctivae. The title of his paper is "Mémoire sur une lésion conjunctivale non encore décrite, coincidant avec l'héméralopie;" finding all his 29 patients with xerosis suffering from night blindness he thought the xerotic coverings of the eye to be the cause of the night blindness as they cut off a certain amount of the light. A. Netter (33) pointed out in the same year (1863) that this view could not be correct because several patients suffering from night blindness did not suffer from xerosis. In the course of time ophthalmologists reached the view that the three ailments, night blindness, xerosis conjunctivae and keratomalacia, were different degrees of the same disease, and this is still the point of view generally accepted.

The relation of the three ailments and especially of night blindness to malnutrition was early suggested. Night blindness often appeared under conditions of bad nourishment, as among prisoners (8), (10) or negro slaves in Brazil (13), or in long sea-voyages (15). Several observations of night blindness among soldiers and prisoners during the war 1914–18 exist (43), (37), (26), (17), (30). The night blindness among orthodox Russians during the Lenten fasts has often been described (5), (22). But several kinds of night blindness exist, and probably but one of these is related to malnutrition, viz., the kind preceding or accompanying xerophthalmia. Quite different in etiology is the congenital night blindess, the night blindness in retinitis pigmentosa, in detachment of the retina, etc. Even the cases among soldiers in the last war have apparently not all had the

same etiology (see C. Augstein (1), F. Best (2), K. K. K. Lundsgaard (26)).

When the relation between xerophthalmia and a deficiency of fat-soluble A-vitamin in the diet had been established by experiments (34), (28) and by clinical observations (32), (6), a similar relation between the deficiency of A-vitamin and the night blindness accompanying xerophthalmia became possible.

In the papers of Mori and of Bloch night blindness of some of the xerophthalmia-patients is mentioned. McCollum (27) and later the authors of the second edition of the Medical Research Council Report on vitamins (29) have compiled observations suggesting the association of night blindness in human beings with starvation for A-vitamin and have mentioned the use of liver and of cod-liver oil as remedies for night blindness, these foods containing much A-vitamin. This treatment of night blindness is mentioned by many authors (1), (10), (13), (17), (21),(39), (40), (43). O. Blegvad found night blindness in 37 out of 66 Danish xerophthalmia patients more than 3 years old and has given a summary of the question (4).

But unanimity about the importance of malnutrition in the etiology of night blindness does not exist. Some authors think alcoholism rather than malnutrition is the main cause (42), (24), (25). More weighty are the arguments in favor of the view that night blindness depends on exposure to the bright sunlight. As early as 1859 Alfr. Graefe (14) observed prisoners suffering from night blindness but recovering through a stay in darkness and relapsing by exposure to light. Several authors agree with Graefe on the basis of similar observations and consider such exposure as the main or the only cause of this kind of night blindness (10), (2), (33), (36). This view is corroborated by the occurrence of most cases of night blindness in the early spring.

Clinical observation can not settle the question of the etiology of night blindness preceding or accompanying xerophthalmia and experimental research work must be done. Experiments concerning the etiology of night blindness do not exist as far as we know.

II. Night blindness and the visual purple. The most obvious experimental treatment of the question as to the etiology of night blindness would be to try if night blindness occurs in animals on a diet deficient in A-vitamin.

It is difficult to demonstrate night blindness in animals, although it is not impossible. Night blindness consists in a difficulty or an impossibility of adapting the faculty of vision to very faint illumination. Carl Hess (16) succeeded in experimenting on this adaptation of the vision in birds. Experiments on the possible relation of night blindness to starvation for A-vitamin have to be conducted on animals whose dietary require-

ments, especially concerning the A-vitamin, are well known. In consequence we had to use rats.

It is more difficult to experiment on rats than on birds in order to examine the adaptation of the vision to faint illumination. Later one of us succeeded in doing so (published in another paper), but in the series of experiments described in this paper we have followed another and more indirect course.

According to a view accepted by many ophthalmologists, night blindness depends on an abnormality in the rod cells of the retina. This anomaly is considered to be connected with the visual purple, situated only in the rod cells. The view is often called the duplex theory of the faculty of vision. According to this theory, originated by Max Schultze in 1866 (38) before the discovery of the visual purple, the rods of the retina have another function than the cones. The ordinary faculty of vision in daylight and the perception of colors should be the function of the cones but the colorless vision at faint illumination ("twilight-vision") should be the function of the rods. After the visual purple in the rods had been discovered, Parinaud (36) supplemented the duplex theory with the assumption of the twilight-vision being dependent on the visual purple. The color of the visual purple is bleached in the light but regenerated in darkness if the retina is not removed from its situation in the eye.

The duplex theory is not accepted by all ophthalmologists. M. Tscherning (41) has opposed it on the basis of his investigations (a summary of the question is given in (18)). In spite of this we think it for the present legitimate to apply the duplex theory as a provisional starting point of our experiments in presuming the faculty of adapting the eye to vision by faint illumination to be dependent in some way or other on the visual purple in the rod cells of the retina. As a consequence of this theory night blindness is presumed to be correlated with an abnormal function of the visual purple, probably a reduced or failing function (36), (18).

III. Preliminary experimental methods. Our experiments purpose to compare the visual purple in the retina of control rats, receiving an adequate diet, with the visual purple of rats on a diet devoid of A-vitamin but adequate in all other respects. Xerophthalmia will develop in rats subjected to such an experimental diet as described by many investigators (first by Osborne and Mendel (34) and by Freise, Goldschmidt and Frank (11)) and the symptoms are identical with the symptoms of xerophthalmia in human beings (20).

When the visual purple is to be examined in the eyes of the experimental rats, the examination must take place before the development of pronounced xerophthalmia, especially earlier than the occurrence of opacity of the cornea (the reason of this necessity will be explained later). This is why a constant occurrence of xerophthalmia in all the rats on the

diet devoid of A-vitamin is a condition of the experiments. Most investigators have failed to produce xerophthalmia in all their experimental rats on such a diet but only in a certain percentage of them (Osborne and Mendel (35) in 50 per cent), only Agnes F. Morgan (31) got xerophthalmia in all. In our stock of rats we have been lucky enough to realize a constant occurrence of xerophthalmia in all rats on the diet devoid of A-vitamin used by us, when young rats were used for the experiments. The initial symptoms of xerophthalmia developed after four to seven weeks of A-vitamin starvation.

The basal food mixture of the diet had the following composition: 200 grams caseinogen, 30 grams agar, 50 grams autolysed and dried yeast, 470 grams rice starch and 50 grams salt mixture. The caseinogen was purified by repeated washing with hot alcohol and with ether, then heated in thin layers to 105° for 24 hours (as recommended by Drummond and Coward (9)). The agar was powdered and purified by being boiled in 96 per cent alcohol. The autolysed yeast was dried at a temperature lower than 40°C. The rice starch was boiled twice with 96 per cent alcohol. The salt mixture was the mixture recommended in the report of the Medical Research Council (29, p. 14).

To the basal food-mixture was added purified butter fat in the diets of control rats and linseed oil or lard in the experimental diets. The lard and the linseed oil were melted, filtered and heated in thin layers to 105°C. for 24 hours. During the experiments control rats as well as experimental rats were kept in a dark thermostat room without windows at 22°C. (except when purposely exposed to the light). The room was dimly lighted by a "glimm-lamp." Every rat was confined to its special cage, made of iron-wire net. Urine and feces fell down through the meshes of the floor. On one side of each cage was an eating-chamber with a food glass. This construction of the cages prevents the animals from eating the feces and from soiling the food.

The first experiments purposed to compare the amount of visual purple in the retinae of experimental rats on a diet devoid of A-vitamin (basal food mixture + 12 per cent linseed oil) with the amount of visual purple in the retinae of control rats (basal food mixture + 12 per cent butter fat).

In order to estimate the amount of visual purple in the retina of a rat we first tried extraction with a solution of bile salts as indicated by W. Kühne (23). The extraction took place in red light. The method had to be abandoned because only a fraction of the visual purple was extracted in this way. Afterwards we adopted a colorimetric method. The principle of this method is indicated by S. Garten (12). Our way of proceeding was the following: the rat was carried in a dark box from the thermostat room to a photographic red lighted dark-room and narcotized by chloroform (in the first few experiments) or by subcutaneous injection of ure-

thane-solution (in most experiments). The eye-ball was enucleated and opened by an equatorial cut. The retina was dissected and the isolated retina, after washing in water, spread on a white porcelain plate. Then the plate with the retina was taken to a room with subdued daylight and quickly compared with a color scale. In the first experiments the color scale reproduced in the paper of Garten (12) was used. It is possible to complete the colorimetric comparison before the bleaching of the visual purple of the retina begins.

In preliminary experiments (expts. 1 and 2) the amount of visual purple in the eyes of four pied rats, taken directly from the dark thermostat room, was estimated. Two of the rats had for 5 and 6 weeks received a diet, devoid of A-vitamin (basal food mixture + 12 per cent linseed oil). The two other rats were control animals on adequate diet (basal food mixture + 12 per cent butter fat). No distinct difference between the amounts of visual purple in the eyes of experimental rats and of control rats was observed.

We did not think it profitable to continue the experiments in this way. The result of these few experiments showed that a constant and conspicuous difference between the amount of visual purple in the eyes of experimental rats and control rats, all kept in darkness, was not to be found. For this reason the experimental method was altered.

IV. Regeneration of the visual purple and night blindness. Possibly other abnormalities connected with the visual purple than a diminution of its amount existed in rats starved for A-vitamin. If such abnormalities had relations to night blindness in the animals the clinical experience about night blindness in human beings could probably point out the way to be followed in further experiments. As mentioned above, some observations suggest a bearing of the action of bright light on the etiology of night blindness in human beings. Several investigators consider the action of light to be the main cause of night blindness. This view may contain a part of the truth.

The principal symptom of night blindness is the difficulty in adapting the faculty of vision to faint illumination. According to existing observations this difficulty is most pronounced when the eyes of the patients have been exposed to intense light, but after a period of rest in darkness this difficulty becomes much less. Parinaud (36), who originated the theory of the relation between night blindness and abnormalities in the visual purple, pointed out that the difficulty of the patients in adapting their vision to faint light augmented toward evening and was aggravated by exposure to intense light. H. de Gouvea (13) observed night blindness in badly nourished slaves in Brazil and describes how the slaves were unable to see, when returning from their work after sunset, but had no difficulty in seeing when starting in the morning before sunrise, although

it was much darker in the morning than in the evening. Patients suffering from night blindness have told one of us about their more pronounced difficulty in seeing in the evening than in the morning.

Since night blindness is more pronounced after exposure of the eyes to light, the abnormality in the function of the eye causing the night blindness is perhaps more difficult to ascertain in eyes not having been exposed to light. In the introductory experiments the rats had been kept in darkness. The color of the visual purple is bleached in light and regenerated in darkness. If the difficulty in adapting the night blind eye to faint illumination has relation to a defect in the function of the visual purple, this defect might possibly be a difficulty in regenerating the color of the visual purple when the color had previously been bleached by exposure of the eye to light. Although present a defect of this kind might not be found if the animal was examined after a stay in darkness.

On the basis of this reasoning we have examined the regeneration of the visual purple after bleaching in the light in rats starved for A-vitamin and compared the results with the regeneration of the visual purple in control rats on adequate diet.

V. Experiments on the regeneration of the bleached visual purple in pied rats after A-vitamin starvation. The procedure adopted in these experiments was the following: the rat was taken from the dark thermostat room and placed in a big box with white floor, roof and walls. The box was illuminated by a 50-light Nernst-lamp, placed under the roof of the box. In the first experiments (no. 3 to 15) control rats and experimental rats were not exposed to light at the same time. In the later experiments (16 to 27) an experimental rat, kept on a diet devoid of A-vitamin, and the corresponding control rat, kept on an adequate diet, was at the same time placed in the lighted box. After a certain time the animals were narcotized and one eye in each of the rats enucleated. The eyes were opened in a dark-room in red light and the color of the retinae was observed. When the retinae were white without any reddish tint the bleaching of the visual purple had been complete. If so the next stage of the experiment was to examine the regeneration of the visual purple in the remaining eye of each of the two rats. The animals were placed in darkness, and after the lapse of a certain time, the other eye was enucleated in both the rats. After opening of the eyes and dissection of the retinae, it was examined by colorimetry as already described to see to what stage of color the regeneration of the visual purple in the retinae had proceeded during the stay of the animals in darkness, and a comparison was made between the amounts of visual purple regenerated in the retinae of the experimental and of the control rats.

The Garten color scale used in the first experiments proved to be impractical because it contained too many colors. This prolonged the colori-

metric estimation. Quickness was essential because the estimation as mentioned had to go on in subdued daylight. We then prepared a very simple scale, containing only five colors, viz.: 1, deepest red to be found in the retina; 2, tile-red; 3, light red; 4, faint light red, and 5, with a reddish tint. This scale made a very quick estimation possible, the difference between its stages being pronounced. Rats, which had not been in the light but were taken directly from the dark cellar, showed a color of their retina corresponding to no. 1 or no. 2 of the scale.

In introductory experiments the conditions of the total bleaching in the light of the visual purple of the rat had to be determined. It proved to be difficult to bleach the visual purple in the eyes of a living rat, unless the animal were an albino. Pied rats contract the pigmented iris and all rats wink when exposed to intense light. On this account it proved necessary to instill atropin in the eyes of pied rats (1 drop of a 1 per cent solution of sulphate of atropin) and to narcotize them during their exposure to light, in order to prevent the animals from shutting the eyes. Urethane narcosis proved best. For deep narcosis of a 100 gram young rat the subcutaneous injection of 25 to 30 centigrams of ethyl urethane is required. In the experiments 10 to 20 centigrams, giving a light nacrosis, sufficed. During the enucleation of the eye the light narcosis was supplemented by ether inhalation.

In the atropinized and urethanized pied rats the visual purple was totally colorless after two exposures of 15 minutes each in the light box. Between the two exposures there was a pause of 15 minutes. This proved to be better than continual exposure to light for 30 minutes in order to avoid the production of opacities in the cornea and of "visual yellow" in the retina by the action of the light (further particulars especially concerning the production of visual yellow are published in (19)).

In albino rats the atropin treatment was superfluous, because the iris did not contain any pigment, and the bleaching out of the visual purple was completed after 15 minutes' exposure to light in the box.

In most experiments the completeness of the bleaching out of the visual purple was controlled, as mentioned, by enucleating and examining one eye after exposure of the rat to the light. Sufficient experience having been gained, it was in some of the later experiments deemed superfluous to continue this control.

The experimental rats had to be used in the experiments dealing with the regeneration of the visual purple before pronounced symptoms of xerophthalmia appeared, because these symptoms very soon are followed by keratitis; and opacities of the cornea impede the effect of the light on the visual purple. The experimental rats were examined when their weight commenced to decrease and enophthalmus and conjunctival secretion began (about the initial symptoms in xerophthalmia in rats, see (20)).

Eighteen experiments were made on pied rats (expts. 3 to 21). In reality these experiments turned out to be introductory or rather of guidance for the final experiments, because some unexpected difficulties appeared.

In table 1 the results of the first 9 experiments on regeneration of the visual purple are represented as described under the table. Five of these experiments (3 to 7) were made on control rats and 4 on experimental rats (8 to 11), having been starved for A-vitamin 4 to 7 weeks. In the controls the color of the visual purple was completely regenerated after 2 hours' stay in darkness. The regeneration seemed to be a little slower in the

TABLE 1

Results of experiments 3 to 11

COLOR SCALE	1 HOUR	1½ HOURS	1¾ HOURS	2 HOURS
No. 1				
No. 1–2				**6** **7**
No. 2				*5* *8*
No. 2–3				
No. 3	**3**	**4**		
No. 3–4				
No. 4	*9*		*10* *11*	
No. 4–5				
No. 5				
No regeneration				

Each horizontal line corresponds to a number of the colorimetric scale. No. 1 is the most intense red color, no. 5 the faintest.

Each column represents a stay in darkness (after the total bleaching of the visual purple in the light) of the stated duration (1 hour, 1¾ hour, etc.). Consequently the table shows to what extent (to what number of the colorimetric scale) the completely bleached visual purple had regenerated after stay of the rats in darkness for periods of different duration.

Heavy type represents control rats on adequate diet.

Italic numerals represents experimental rats starved for A-vitamin.

The numbers refer to particular experiments.

experimental rats than in the controls but the difference was very trivial and in consequence doubtful.

In the next series of experiments the rats were exposed daily to the light in the period previous to the examination of the regeneration of the visual purple. This was done on the basis of the following supposition: if the experimental rats regenerated the visual purple more slowly than the control rats, the difference could be expected to be more pronounced when the function of regeneration had been "fatigued" by daily bleaching of the purple.

The daily exposure to light was accomplished either by placing the

animals every day for two hours before a window facing the south (expts. 12, 14 and 16) or in most experiments (expts. 13, 15 and 17 to 21) by placing the rats several times daily in the light box. The rats had always atropinized eyes when exposed to the light. The experimental rat and his corresponding control rat were in every case treated exactly in the same manner and exposed simultaneously to the same amount of light in the same way. Previous to the examination the experimental rats had received the basal food mixture + 15 per cent or 12 per cent of lard and the control rats the basal food mixture + 15 per cent or 12 per cent butter fat.

Twenty-four examinations of the regeneration of the visual purple in experimental rats and corresponding control rats were made in this way (expts. 12 to 21). Experiments 12 to 15 comprise each a single rat. In nos. 16 to 21 each number comprises the experimental rat and the cor-

TABLE 2

Representing experiments 12 to 21

COLOR SCALE	1 HOUR	1½ HOURS	2½ HOURS	3 HOURS
No. 1				**18** *19* *19*
No. 1–2			**17**	
No. 2		**16**	*17*	*21* **21**
No. 2–3	*20*			
No. 3	**20**	*12* *14* *16*		
No. 3–4	**17** **18** **19**			*18*
No. 4	*18* *19* *21* **21**			
No. 4–5		*15*		
No. 5				
No regeneration	*13* *17*			

The explanation is given under table 1.

responding control rat. In nos. 17 to 19 and 21 the regeneration was examined in both eyes of each rat.

In spite of all precautions disturbing factors were present in these experiments. In nos. 13, 14 and 15 the experimental rats suffered from keratitis. In experiments 17 to 21 a new disturbing factor appeared. It was observed that during the process of regeneration of the visual purple an adhesion of the pigment from the pigment cell layer to the dissected retina in most cases occurred. This adhesion of the pigment did not take place as was to be expected from statements in the literature either in the bleached retina or in the retina of rats taken directly from the dark cellar. The adhesion only occurred during the regeneration of the visual purple after complete bleaching of its color in the light. The phenomenon occurred in experimental rats as well as in control rats. The adhesion of the pigment imparts to the retina a brownish color and often makes the

colorimetric estimation of the amount of visual purple very difficult and in consequence uncertain.

In spite of these defects connected with experiments 12 to 21, the results are represented in table 2. On the whole the regeneration of the visual purple is slower and—during the time of observation—more imperfect in the retinae of the experimental rats than in the retinae of the control rats. In the control rats the regeneration was complete after $1\frac{1}{2}$ to $2\frac{1}{2}$ hours' stay in darkness.

To get a better survey over all the 33 regeneration examinations in the 18 experiments, 3 to 21, the average results have been computed in table 3. The way of computing was the following: the amount of visual purple regenerated after each given period of stay in darkness was estimated in several experiments; in each estimation the amount of regeneration was expressed by the number of the color scale, matching the color of the

TABLE 3

Thirty-three estimations in pied rats of the regeneration of the bleached visual purple (from expts. 3 to 21).

Average numbers from the color scale after regeneration in periods of different duration. The number of rats from the observation of which the numbers are taken is given.

	EXPERIMENTAL RATS	CONTROL RATS
1 hour regeneration	4.6 (7 rats)	3.4 (5 rats)
$1\frac{1}{4}$ hours' regeneration		3.0 (1 rat)
$1\frac{1}{2}$ hours' regeneration	3.6 (6 rats)	2.0 (1 rat)
2 hours' regeneration	2.0 (2 rats)	1.3 (3 rats)
$2\frac{1}{2}$ hours' regeneration	2.0 (1 rat)	1.5 (1 rat)
3 hours' regeneration	2.2 (3 rats)	1.3 (3 rats)

retina (the simplified color scale containing five colors) after, say, one hour's stay in darkness, seven experimental rats were examined and seven values for the amount of regeneration of the visual purple in a period of this duration was accordingly ascertained, these values being represented by numbers; the average of these 7 numbers is 4.6 and this value of 4.6 is taken as representing the average amount of regeneration of the visual purple after one hour's stay in darkness after the complete bleaching of the purple. In the same way the number 3.4 is found to represent the average amount of visual purple regeneration in 5 control rats after 1 hour's stay in darkness, and so on. It is to be remembered that no. 1 means the deepest red color and no. 5 the faintest. Number 6 is taken as representing no regeneration at all.

These average scale numbers for the regeneration of the purple after periods of varying duration are put down in table 3 and represented as a

diagram in figure 1, the ordinates corresponding to the average numbers of purple regeneration, the abscissae to the corresponding duration of the stay in darkness.

Table 3 as well as figure 1 shows how the regeneration of the visual purple on an average proceeds slower in the experimental rats than in the control rats. Want of A-vitamin in the diet seems to make the regeneration of the visual purple slower.

But the results are not decisive, partly because the "average error" of the results is very great, partly because disturbing factors had been present, especially the fastening of pigment to the retina.

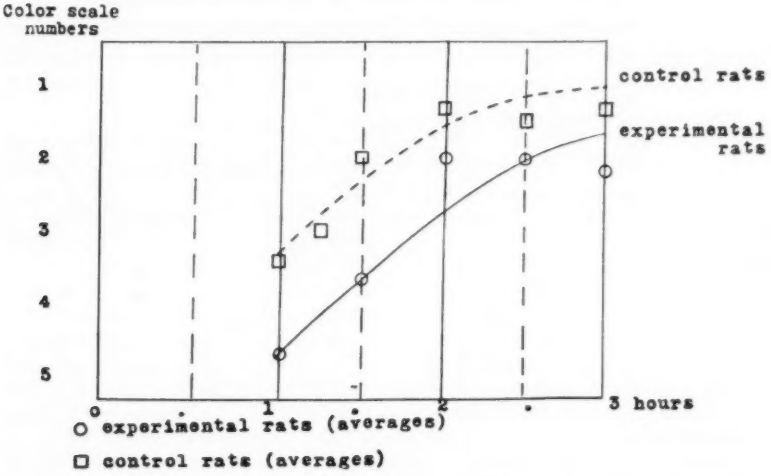

Fig. 1. Diagram of the regeneration of the visual purple in experimental and control rats.

VI. Experiments on albino-rats. The difficulty in the colorimetric estimation dependent on the adhesion of pigment from the pigment cell layer to the dissected retinae can be avoided in experiments on albino rats. No visible pigment is to be found in the pigment cell layer or in the iris of the albinos. This last makes the instillation of atropin during the exposure to light superfluous.

In the albinos 22 estimations of the regeneration of the visual purple after complete bleaching were made in 12 rats (expts. 22 to 27). In these experiments advantage could be taken of the experience regarding disturbing factors gained during the experiments on pied rats. Accordingly the main features of the experimental method were the following: *1.* The experimental rat and the corresponding control rat had the same

weight, when the feeding with the special diet commenced. The experimental rats got the basal food mixture + 15 per cent lard, the control rats the basal food mixture + 15 per cent butter fat. *2.* In the period previous to the examination of the visual purple all rats had daily been exposed to the action of light by being placed for 6 hours in a window. An experimental rat and the corresponding control rat were always placed in the same window during the same time. *3.* The regeneration of the bleached visual purple in an experimental rat was examined as soon as the rat commenced losing in weight before the occurrence of other xerophthalmic symptoms than enophthalmus and conjunctival secretion. The regeneration was in every case examined in the control rat simultaneously with the examination of the corresponding experimental rat. *4.* The bleaching of the visual purple preceding the examination of its regenera-

TABLE 4

Representing experiments 22 to 27 in albino rats

COLOR SCALE	¾ HOUR	1½ HOUR	2 HOURS	2½ HOURS	3 HOURS	4 HOURS
No. 1					**25 26**	
No. 1–2			**22 25 26**			**27**
No. 2					**27**	
No. 2–3					**24**	
No. 3	**22**			*22*		
No. 3–4			**23**			
No. 4	*22*	**23**				*27*
No. 4–5			*23*			
No. 5			*25*		*24 25 27*	
No regeneration		*23*	*26*		*26*	

The explanation is given under table 1.

tion was always complete. Twenty minutes' exposure to the 50-light Nernst-lamp in the light box sufficed in narcotized albino-rats. The narcosis was effected by the subcutaneous injection of 10 centigrams of ethyl urethane in aqueous solution, supplemented by ether inhalation during the enucleation of the eyes.

The results of the 22 examinations in albino rats are represented in table 4. From this table it is evident that the regeneration of the bleached visual purple is much slower and—within the time of observation—much more incomplete in the experimental albino rats, starved for A-vitamin, than in the control rats on an adequate diet. After 3 to 5 hours' stay in darkness the visual purple was almost completely regenerated in the retinae of the control rats, but very little color had been regenerated in the retinae of the experimental rats. In table 5 the average amounts of regenerated visual purple at different points of time have been computed on the basis

of the corresponding numbers of the color scale in the same way as in table 3. The difference between experimental rats and control rats is evident. But the main point in the results of the albino experiments is that a pronounced difference not only appears between these averages but between every experimental rat and its corresponding control rat.

From these results we think it warranted to conclude that starvation for A-vitamin in rats produces a defect in the function of the visual purple, the defect consisting in a slowness in the regeneration of the visual purple after its having been bleached through exposure to intense light.

VII. EXPERIMENTS ON THE REGENERATION OF THE BLEACHED VISUAL PURPLE IN RATS STARVED FOR B-VITAMIN. It may be asked if the ascertained defect in the regeneration of the visual purple is something exclusively connected with starvation for A-vitamin, or if other dietary defects are able to produce the same result. A thorough investigation of this

TABLE 5

Twenty-two estimations in albino rats of the regeneration of the bleached visual purple (exps. 22 to 27)

Average numbers from color scale after regeneration in periods of different duration.

	EXPERIMENTAL RATS	CONTROL RATS
¾ hour's regeneration	4.0 (1 rat)	3.0 (1 rat)
1½ hours' regeneration	6.0 (1 rat)	4.0 (1 rat)
2 hours' regeneration	5.2 (3 rats)	2.0 (4 rats)
2½ hours' regeneration	3.0 (1 rat)	
3 hours' regeneration	5.2 (4 rats)	1.6 (4 rats)
5 hours' regeneration	4.0 (1 rat)	2.0 (1 rat)

question would require many experiments. We made only two experiments on rats receiving a diet adequate in respect to A-vitamin, but deficient in B-vitamin (expts. 28 and 29). In these experiments, performed in the way described, no difference was observed between experimental rats and control rats concerning the regeneration of the bleached visual purple. In all the visual purple was completely regenerated after 2 hours' stay in darkness.

DISCUSSION OF THE RESULTS. A deficiency in the diet of rats of the fat-soluble A-vitamin produces a series of well-known symptoms, the most characteristic being the effect on growth and the eye disease xerophthalmia. In addition to the known symptoms of this deficiency our experiments have pointed out another symptom, viz., a delay in the regeneration of the visual purple of the retina, succeeding the bleaching of the purple in the light. This symptom is an early one, being manifested as soon as the

growth of the young rat stops and earlier than the onset of pronounced xerophthalmic symptoms.

In human beings a relation exists between xerophthalmia and a certain kind of night blindness, the night blindness often preceding the xerophthalmia. Some authors have suggested a relation of this kind of night blindness to a deficiency of A-vitamin in the diet, but others have made observations suggesting a connection between night blindness and exposure of the eyes to intense light. If this kind of night blindness depends on a defect in the function of the visual purple, identical with that observed in the rats starved for A-vitamin, both of these views contain a part of the truth.

In rats a deficiency in the diet of A-vitamin produces a defect in the faculty of regenerating the visual purple after bleaching of the purple in the light. But this defect is only to be observed after the eyes of the rats have been exposed to the action of intense light. The night blindness behaves in the same way. The ailment is presumably caused by an identical deficiency in the diet, but the symptom, the night blindness proper, is only manifest after exposure of the patients' eyes to intense light. This analogy suggests the relation of night blindness to a defect in the function of the visual purple identical with that here reported for rats.

SUMMARY

1. Methods of estimating the amount of visual purple in the retinae of rats by colorimetry are described. The conditions of complete bleaching of the visual purple in eyes of living rats by action of the light are investigated. Methods of examining the regeneration of the bleached visual purple are described.

2. Rats starved for A-vitamin and corresponding control rats receiving an adequate diet are examined as to the amount of visual purple in their retinae and their faculty of regenerating the color of the bleached visual purple.

3. No influence of starving for A-vitamin on the amount of visual purple in the retinae of rats kept in darkness has been found.

4. When the visual purple of the retinae has been completely bleached by exposure of the rats to light, the regeneration of the purple is delayed in rats starved for A-vitamin as compared with control rats receiving an adequate diet. This abnormality is much more pronounced in albino rats than in pied rats. In pied rats a fastening of the pigment from the pigment cell layer to the dissected retina impedes the examination of the visual purple. Previous to the examination of the regeneration of the bleached visual purple, the rats have to be daily exposed to the light. This has to be done in the same way in experimental rats and control rats.

5. The abnormality in rats starved for A-vitamin as to the regeneration of the bleached visual purple occurs earlier than pronounced symptoms of xerophthalmia.

6. The abnormality in the regeneration of the bleached visual purple has not been found in rats starved for B-vitamin.

7. In human beings a relation between xerophthalmia and a kind of night blindness, often preceding the xerophthalmia, has been suggested by several authors. This kind of night blindness is by some authors thought dependent on a deficiency in the diet of A-vitamin, by others on the exposure of the eyes to intense light. If this kind of night blindness depends on a defect in the function of the visual purple, identical to that observed in rats starved for A-vitamin, both views contain part of the truth.

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DEMONSTRATION OF HEMERALOPIA IN RATS NOURISHED ON FOOD DEVOID OF FAT-SOLUBLE-A-VITAMIN

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That it has long been possible to produce keratomolacia in different animals, especially in rats, is well known. It has further been possible in rats to produce the same exterior eye symptoms as in human beings suffering from xerophthalmia (first observed by McCollum, and described more minutely by the author), except, owing to physical conditions, Bitot's spots. On the other hand, eye symptoms appear in rats which are not found in human beings suffering from the same disease, namely, enophthalmus, loss of hair at the cilia edges, and a peculiar reddish-brown secretion. In rats, enophthalmus is a constant and very pronounced symptom which appears very early, generally at the time the young rats stop increasing in weight. In man, however, another early symptom exists, namely, hemeralopia which as a general rule can be demonstrated only in cases where nutritive disturbances occur in adults. Frequently this is the only symptom found; sometimes, however, xerosis and Bitot's spots are found at the same time.

Hitherto, indications have not existed regarding the fact whether hemeralopia may also be found in rats nourished on food devoid of fat-soluble-A-vitamin, but from experiments made by L. S. Fridericia and Ejler Holm (see paper just preceding), this may be inferred. It was found that regeneration of the visual purple after bleaching takes place considerably more slowly than in normally nourished rats. The retinae of the experimental animals were colorless or nearly colorless when the animals, after exposure to light, had stayed in the dark for two or three hours, while the retinae of the control animals were of a deep purple under the same conditions. The experiments succeeded fully only in the case of albino rats.

At that time no attempt at any direct demonstration of hemeralopia was made, because a reliable direct demonstration of hemeralopia in these animals was considered to be very difficult. At Professor Fridericia's suggestion I later took up the problem.

The best-known physiological researches concerning hemeralopia in

animals are those made by Hess (Arch. f. Augenhk., 1907) on birds. Hess tested partly such birds as are supposed to see badly in weak light, namely, poultry and pigeons, partly night-birds, such as owls. He tested the hens by making them pick up grains in a light which could be regulated with an Aubert's diaphragm behind which was placed a dull glass plate. He then watched how much he could diminish the opening in the diaphragm before the hens stopped eating. He found them to be able to adapt their faculty of vision to darkness, for, after a stay in the dark, they would eat in a light less intense than that in which they stopped eating without the previous exposure to darkness. On the other hand, they stopped eating before the grains became invisible to himself. The owls were made to peck at a piece of meat which was held before them, and he found that they stopped pecking at it when the light became so feeble that the meat also became invisible to himself.

The methods used by Hess are not suitable for the demonstration of hemeralopia in rats because, partly, these animals are very restless and, partly, because they can help themselves by means of their whiskers and their sense of smell. Finally, the experimental food without fat-soluble-A-vitamin which is given them is not very attractive to them. That was, amongst others, the very reason why it was considered hopeless to attempt to train them to find special food vessels and thus to test their visual power. This method was used by Jellinek (Arch. f. mikr. Anat. u. Entw., 1923, xcix) in order to ascertain whether a rat can see but, in my opinion, it is not satisfactory. What appeared more suitable was a method used by Ch. Waugh (1910) to test the visual power of mice, and employed by Koppányi (who, like Jellinek, worked in Przibram's laboratory) in order to examine whether rats could regain their faculty of vision after amputation and replantation of the eyes. Koppányi placed the rats on a pedestal in a large cage. In the cage there was also a nest into which the rats would jump as soon as they were placed on the pedestal. The higher this pedestal the longer it would be before they could make up their minds to jump. Again, the blind rats remained sitting still or else climbed carefully down the pedestal. Koppanyi's result were but little convincing, especially on account of his statement that the rats could see with eyes, which, according to simplest ophthalmological conceptions, must have been absolutely useless. Probably, Koppányi's rats had become so well oriented during the time which elapsed before they, in his opinion, should have been able to see again that they were able to jump down without the aid of sight.

In the winter 1923–24 I tried to demonstrate hemeralopia by means of a similar "jumping test" in young rats nourished on food devoid of fat-soluble-A-vitamin. The rats were tamed from infancy, as in my later experiments, so that they were easy to manage. The experiments

were made at that stage of the disease where they had stopped increasing in weight and where enophthalmus had set in, although, as yet, no corneal affection had appeared. Their pupils were constantly kept atropinized, and they were daily placed in a light-box for some time. After at least 15 minutes' stay in this box they were brought down into the dark cage where they were tested with different intensities of light. The test was made in the following manner: The rat was placed on a small board which was held by the experimenter and could be moved to a distance above the floor, or to different distances above a table as desired. This way of testing proved to be unsuitable, because the rat would sometimes have long "inactive" spells when it did not jump down at all, even if it could, and, at other times, it was so impatient that it always jumped down. Moreover, the influence of light was uncertain, for the sick rats would close their eyes very tight in the light-box; urethane narcosis, such as is used for the determination of visual purple could of course not be used here.

Some experiments on normal rats showed that these animals have a highly developed faculty of adaptation to darkness. In a room in which I could not myself see at all even after half an hour's stay in the dark, a rat could, under the same conditions, jump readily from a table onto a piece of white paper placed on a shelf at the same level as the table, but at half a meter's distance away.

My final method of testing was as follows:

In order to work with the strongest light possible the experiments were performed in the summer. The importance of such procedure was indicated by our knowledge of hemeralopia in man and by our experience in bleaching the visual purple as described in the preceding paper.

The rats were placed on a table before a window, with direct sunshine from about 9 a.m. to 1 p.m., and, as no albinoes were employed, the pupils of the rats were dilated now and again with atropin. Moreover, a stay in the light-box immediately before the test was avoided, instead of which arrangements were made so that the tests could always be made from 2 to 3 p.m. Absolute uniformity of illumination could of course not be obtained owing to the varying light and because of the rats' varying liveliness and general conduct.

I therefore gave up the jump-test. During the previous experiments I had noticed how eager the rats were to get away from the table where they did not find any covering and how dexterous they were in jumping in a horizontal direction onto a shelf, where they liked to hide themselves under some cages. Therefore, the rats were now placed on the table, one corner of which was turned towards the shelf, thus enabling the rat at one bound of 10 cm. length to reach the top of a cage placed on the shelf. A piece of white paper was placed on the cage which stood on a tray, where there were several other objects under which the rats could hide. When the

rat was able to see, it ran at once to the right corner and jumped over, otherwise, it would run from one corner to the other, stopping at each corner, and trying to take a leap. Frequently, it seemed to be aware which corner was the right one but did not venture to leap; however, as soon as a stronger lamp was lit (see later), it would immediately jump over. This method had the advantage that the rat which was eager to jump, had the four corners to choose from and, moreover, it did not matter so much if it stopped to trim itself or fell into inertia, for then the time was not measured, but a note was only made every time the animal ran toward a corner to spy.

The rats were tested one at a time, at first with an illumination with a 10 candle-power bulb placed about 1 m. above the table. The rat would always jump over quickly, especially when it had previously been allowed to stay a little while on the shelf. It would run straight to the corner and leap over. After that it was again placed on the table, the light was extinguished, and now the room was only illuminated by an electric lamp which was shaded with blue and black tissue paper, to such an extent that the above-mentioned white paper on the cage, onto which the rat was to jump, became invisible to me when looking directly at it. However it remained clearly visible when I looked to the side. With this illumination I was able to see everything in the room clearly, thus I could observe the movements of the rat and was able to make notes on a piece of paper. However, it was impossible to perceive colors. This illumination corresponds to that in which persons suffering from hemeralopia become blind; with a little stronger light, where red colors can still be perceived and the fovea still functions, as for instance, with clear moonlight, these patients can still manage to find their way. After having extinguished the stronger light, I lifted the table, turned it around a couple of times, and placed another corner of it toward the cage, at the same distance from, or a little nearer it. *Now the difference between experimental-rats nourished on food lacking fat-soluble-A-vitamin and normally nourished control-rats became evident,* the latter jumping over at once, while the former, when they had for some time been nourished on food without fat-soluble-A-vitamin, would run round from corner to corner, perhaps stop at the right one, run for a leap, but would give it up again. As a general rule they would jump at last, but in several cases the jump was made only after the lamp was lighted, but then very promptly. In a few instances, one of the rats nourished on food without fat-soluble-A jumped off the wrong corner and fell on the floor, and I noticed that it did not jump down in the manner habitual to rats when jumping downwards, but in a markedly forward-going direction.

A few special tests were made. Thus, in the beginning, one of the normally nourished control-rats was not placed in the light, but was left

all day in the cage, because I thought it possible that a slight "hemeralopia" might have developed in the other control-rats owing to their stay in the light. However, this did not manifest itself during the experiments. On one occasion, all the rats were placed in the stable, and it appeared afterwards (24/6) that there was not a trace of hemeralopia in any of them. That there could be demonstrated hemeralopia the next day in but one single of the experimental-rats nourished on food without fat-soluble-A-vitamin, was probably due to the test being made on the following day 2 hours later than usual. On the 26th of June there was again distinct hemeralopia in all of them.

The hemeralopia in the experimental-rats could be detected soon after the alimentation on food without fat-soluble-A had begun, that is to say, after a lapse of about three weeks, *at a stage where it was impossible to perceive any other sure signs of avitaminosis* except a slight failure to increase normally in weight. Thus, they had not yet arrived at the stage where the increase in weight ceases, this period usually beginning after the lapse of about 4 weeks, when the other changes also usually manifest themselves. In the course of the following week, however, enophthalmus became quite evident, and the rats became less lively. The experimental rats were frequently observed sitting still, with half-closed eyes turned away from the light, while the control rats moved freely about. *Finally, the experimental rats were nourished on the same food as the control rats,* at first two of them, and, a few days later, the third also. It appeared that, *2 to 3 days after the change of nourishment, the hemeralopia ceased.* The light was just as bright but the rats seemed livelier, their eyes being more open, while they stayed in the light.

In my opinion, the experiments have proved that hemeralopia can be produced in rats under the same conditions as alimentary hemeralopia in man. Hemeralopia does not develop through lack of fat-soluble-A-vitamin alone; *it is necessary also that the individuals be much exposed to light.* In animals which live in a dark stable, no hemeralopia appears, and in some experiments made in the winter months when the animals, on account of the cold, could not be left before the window, there was no hemeralopia to be detected even though the retina had been bleached owing to the stay in a lit-up box.

As in alimentary hemeralopia in man, I found in rats a delayed adaptation to darkness, but no lack of adaptation. The functional state in this hemeralopia is presumably the same as that which, normally, though for a shorter period, is found in persons who, after having been exposed to a strong light, come into a dark room.

No observations have been made as to whether, at later stages of the disease, an absolute hemeralopia with an essential limitation of the adaptation can develop; the entire bodily condition of the rats then becomes so

bad and their dread of light so great that it probably could not be done. Owing to these obstacles soon becoming evident and insuperable, it seemed useless to try to show degrees of hemeralopia during the development of the diseased condition. A similar obstacle was also at hand in changing conditions of daylight.

The experiments show furthermore that the hemeralopia appears very early during the avitaminosis, as is also seen in man.

Finally, the experiments indicate that the eyes of the rats behave about like human eyes with respect to the boundary between night-sight and day-sight or, in other words, with respect to the point the uvea begins to function.

THE PASSAGE OF WATER THROUGH THE SKIN OF THE FROG, AND THE RELATION BETWEEN DIFFUSION AND PERMEABILITY

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I. INTRODUCTION. In living units the transport of materials is concerned only with the transport of liquids, for most or all materials are in water solution. The transport of materials in the gaseous or liquid state constitutes the general phenomenon called diffusion, and the physical forces with which this phenomenon is correlated are fairly well understood. Though we must distinguish frequently between solvent and solute as regards diffusion, such distinction depends for the most part upon the peculiar properties of liquid water. The importance of the physical phenomenon of diffusion in the living economy has been incorrectly estimated, however, in two ways; on the one hand by those who assume without evidence that secretion and absorption are nothing but diffusion; and on the other hand by those who suppose that because a living tissue is capable of doing work in transporting certain materials, that diffusion in the physical sense does not occur.

Due to the heterogeneous nature of all living units, diffusion never occurs on an observable scale without the intervention of phase boundaries or membranes, the general effect of which, as will be shown below, is to intensify certain types of diffusion, and to nullify such phenomena as convection.

The physiologist's chief interest concerning transport of materials must therefore be in systems containing membranes. In addition to studies of artificial membranes, data have been recorded by numerous investigators upon two general types of living membranes; multicellular tissues which are spread in two dimensions, such as skin, mucosa and mesentery, and smaller protoplasmic units such as protoplasts, unicellular organisms and blood cells. It seems obvious that to analyze what takes place when materials pass through living membranes, one must know to what extent and by what means they pass through artificial membranes. With this intent we (1) have studied carefully the diffusion *of water* through collodion

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membranes. We found, in common with other investigators, that two sets of forces are at work in these artificial systems,—namely, osmotic and electrostatic. The magnitude of these forces varies primarily with the materials dissolved in the water; the membrane serves merely to intensify under certain conditions the osmotic or electrostatic diffusion potentials. We found that solutions which are characteristic of living tissues, do not themselves confer forces of significant magnitude upon artificial membranes, and diffusion of water is always a surprisingly slow process. Only in systems having large membrane surfaces and small phase volumes can interchanges proceed rapidly between physiological solutions, without the interjection of other energy than that which accompanies differential distribution of materials. We believe that both these conditions are fulfilled in all living tissues.

Our next step in considering the exchange of water through membranes is to compare accurately its passage through an artificial and through a living membrane. For this purpose we have used the skin of the frog *Rana pipiens*. Certain observations could be made upon the living frog, while others could be made only upon isolated frog skin; both kinds of material were therefore used.

The passage of water through the skin of intact frogs was studied by weighing the frogs at intervals during their immersion in various solutions. When it was desired to eliminate individual differences in reaction to these media, groups of 3 frogs were weighed together.

Isolated frog skin was studied by pithing frogs without anesthesia, and removing the skin of the trunk region. The isolated skin will survive for 16 to 90 hours after its removal from the body. This can be ascertained by measuring its oxygen consumption, as was done by Abderhalden and Wertheimer (2), by measuring its electrical resistance as Schwartz (3) did, by measuring the electrical potential difference which its two sides maintain as DuBois Reymond (4) did, or merely by noting the large change in rate of passage of certain materials through it which occurs at "death," as will be described below.

Short glass tubes 17 mm. in diameter were covered at one end by a piece of the fresh frog skin, which was tied in a stretched condition by a rubber band. The tubes were closed at the other end by perforated rubber stoppers containing manometer tubes 3 mm. in internal diameter. Various solutions were placed inside, and the tubes were then immersed in beakers of another solution. Each experiment started with the water level slightly higher inside than outside, to compensate for capillarity in the manometer tube which amounted to about 9 mm.; and changes in water level were followed continuously. The frog skin systems were set up in pairs, one with the skin in its normal orientation, the other with the skin (usually from the same frog) inside out, or with its morphological

outer surface bathed by the solution inside the glass tube. The same solution was placed inside and the same solution outside for both of a pair. The inner solution was about 10 cc. in volume; the outer about 500 cc. contained in a 600 cc. beaker.

The collodion membranes were of two kinds. In the one, collodion solution was molded into 50 cc. sacks, as previously described (1). In the other, collodion solution was poured on a clean glass plate and allowed to dry in a large sheet. Before it was completely dried it was removed from the glass surface, and small pieces of it were tied over the ends of glass tubes which were 17 mm. in diameter. The tied edges were trimmed down and cemented firmly to the tubes with more collodion solution. Then the membranes were immersed in water, and bathed inside with a one per cent solution of gelatin for 24 hours before being used.

II. Experiments with isolated frog skin. The rate at which dissolved materials pass through a membrane, either artificial or living, is usually considered to depend in the first instance upon the "permeability" of the membrane to them. In the following experiments it must be borne in mind that a piece of frog skin is not under "normal" conditions so long as its inner surface is not bathed by lymph and its outer surface by pond water, and its nerve and blood supply intact. Such absence of normal conditions is known frequently to change a tissue's "permeability."

The permeability of *non-living* membranes is but partially understood. We know that such membranes do not act merely as mechanical sieves in the sense of Traube (5). In small pores certain "capillarity" effects occur, as was long ago postulated by Fischer (6). These effects are due to electrostatic charges, as was conclusively demonstrated by Perrin (7); and it appears to be because of these omnipresent charges that such membranes may be relatively "semipermeable" (8). The semipermeability is consequently *not* a mere expression of the fact that solute particles are larger than the molecules of water, even in the case of non-electrolytes, for the diffusing particles are ordinarily much smaller than the spaces between the smallest units of the membrane. It is at present widely believed, therefore, that a collodion membrane acts as a sieve, not by mechanically holding back solute particles, but by repelling them electrostatically.

It is impossible to describe the permeability of any membrane except in quantitative terms and with reference to the penetrating substance. Collodion membranes are penetrated to some degree by every sort of water-soluble substance we have tested. The rate of penetration of hemoglobin and gelatin is very slow, that of sugar and sodium chloride more rapid, and that of water under hydrostatic pressure very rapid. But each rate of penetration depends for its comparative evaluation upon the standardization of conditions which are incompletely understood, for

not only must the membrane be mechanically porous, but the varying "capillary" conditions must be favorable to the penetration. It seems probable at the present time that "permeability" will turn out to be entirely a summation of the several electro-capillary conditions, which depend in great part upon the solution present in the membrane pores. For the present problem it is merely necessary to know that under all conditions the collodion membranes were mechanically capable of allowing

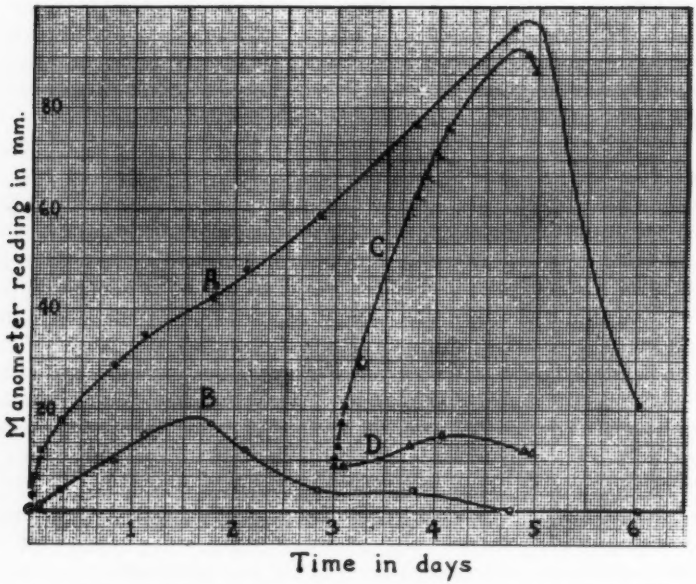

Fig. 1. The actual time courses of passage of water through frog skin from tap water into salt solution. Area of skin = 2.9 sq. cm. *A* and *C*, skin with outer surface in tap water. *B* and *D*, skin with inner surface in tap water. In *A* and *B* the experiment began at 0 time and the water flowed into Locke J; in *C* and *D* the experiment began at time 3 days and the water flowed into Ringer G. The influence of skin orientation is pronounced, and lasts several days.

water to penetrate faster than any substance dissolved in it. For this reason it was possible, within certain limits, to study the passage of water as though the solutes were not diffusing meanwhile.

The passage of water through any membrane takes place in several chronological stages. These we may distinguish by following the time courses of several experiments with living membranes. In figure 1 is shown, over periods of several days, the progress of four experiments with four different preparations of frog skin. In the cases where water passes

through the frog skin at all, it passes at first with considerable rapidity, and then more slowly. In the course of time the direction of flow may reverse and ultimately come to hydrostatic equilibrium. In figure 2 we have a more minute analysis of the time courses, and it can be seen that within the first hour there are initial adjustments in water equilibrium, which may be independent of later events.

It is evident from figures 1 and 2 that we can, depending upon the time period chosen, study *a*, initial adjustments; *b*, steady rate of passage; *c*,

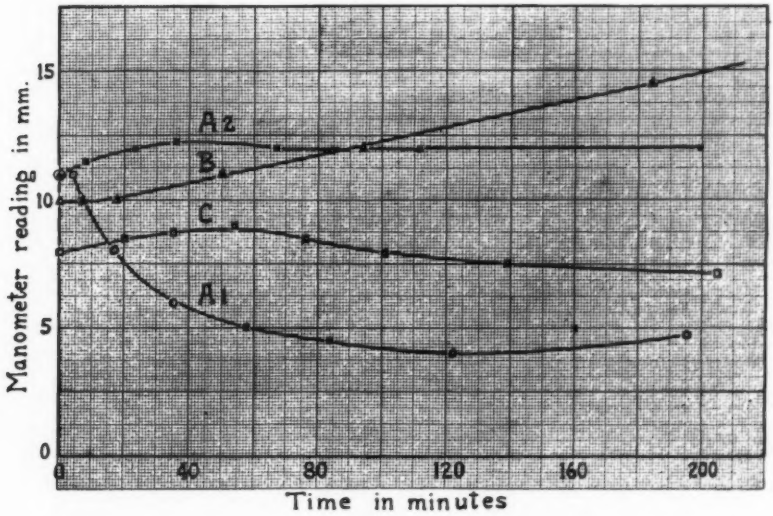

Fig. 2. Initial time courses of passage of water through frog skin from tap water into 0.040 M KCl solution. A_1 and A_2, skin with outer surface in tap water; *B*, skin with inner surface in tap water; *C*, skin with inner surface in Ringer G. Curves A_1 and A_2 represent the same piece of skin in successive tests, showing that water flows away from the KCl solution only initially. The influence of skin orientation is seen by comparing curve *A* with curve *B;* of chemical environments of inner surface by comparing curve *B* with curve *C*.

osmotic equilibrium, if any, or *d*, recovery of hydrostatic equilibrium which probably consists chiefly in the diffusion of solutes after a change in "permeability." We propose to limit the present study to the first two periods, namely, the initial adjustments and the steady rates of passage of water. These are the processes in which the rôle of non-living membranes is most controllable; and these two periods yield the chief points of comparison between living membranes and non-living ones.

The results obtained in all of the experiments which we have thus far carried out with freshly isolated frog skin under standardized conditions

TABLE 1

Passage of water through 2.9 sq. cm. of frog skin, in terms of millimeters rise of water in a 3 mm. manometer

In each case a positive number indicates flow away from pure water (in A and B) or Ringer's solution (in C). Temperature 18° to 23°C.

SOLUTE	MOLAR CONCENTRATION	A OUTER SURFACE BATHED IN TAP-WATER			B INNER SURFACE BATHED IN TAP-WATER			C INNER SURFACE BATHED IN RINGER G*		
		First hour	Second hour	Third to twenty-sixth hours	First hour	Second hour	Third to twenty-sixth hours	First hour	Second hour	Third to twenty-sixth hours
Tapwater		0	0	0						
Tapwater		0	0		2	0		0	0	
Ringer F*	0.115							0	0	0
Ringer G*	0.109	0	0	6	3	3	56	0	0	0
Ringer H*	0.119	0			6	3½		2	0	
Locke J*	0.163	1	½	13	8	4	21	0	0	0
Locke J*	0.163	3	2	14	1	5	23			
NaCl	0 040	−½	½		2½	2½		−1½	0	
NaCl	0.040	−2	0							
NaCl	0.040	−3	0							
NaCl	0.240				1	3	58	0	0	−2
NaCl	0.240				11	4½	38			
NaCl	0.500	−½	−2½							
KCl	0.020	−4	0							
KCl	0.020	−3	0							
KCl	0.040	−6	−1		2½	1½		½	−1	
KCl	0.040				1	1½	12			
KCl	0.060	−3½	0		2½	2		1½	0	
KCl	0.060	−3	−1		5½	3½		0	0	
KCl	0.120	−1½	−½		3	2½	43	2½	½	0
KCl	0.120				4	5	69			
KCl	0.300	0	0		4½	4½				
$CaCl_2$†	0.014	−1	0	2	2	1	9			
$CaCl_2$†	0.052	2	2	17	2	2	14			
Na_2SO_4	0.004	0	0	2	1½	½	5			
Na_2SO_4‡	0.031	−3	−1		11	5				
Na_2SO_4	0.031	−2			2					
Glycerine	0.050	−2	0		3	0				
Sucrose	0.050				3	0				
Sucrose	1.000	8	7		5	6				

* Ringer F = 0.111 M NaCl, 0.002 M KCl, 0.001 M $CaCl_2$.
Ringer G = 0.103 M NaCl, 0.0014 M KCl, 0.002 M $CaCl_2$, 0.002 M $NaHCO_3$.
Ringer H = 0.086 M NaCl, 0.0027 M KCl, 0.002 M $CaCl_2$, 0.027 M $NaHCO_3$. This solution formed a precipitate, though recommended by Hamburger (34).
Locke J = 0.154 M NaCl, 0.0056 M KCl, 0.002 M $CaCl_2$.

† Plus 0.0004 M HCl.

‡ Plus 0.0006 M NaOH.

are given in table 1. We have in each experiment plotted the complete time curve, and then read off the rate of water passage over different periods. In the table we give *1*, the initial adjustments of the first hour; *2*, the beginning of steady passage of the second hour (in which period the passage is usually most rapid); and *3*, the total passage in the subsequent 24 hours. Most of our considerations are based upon the rates for period *2*.

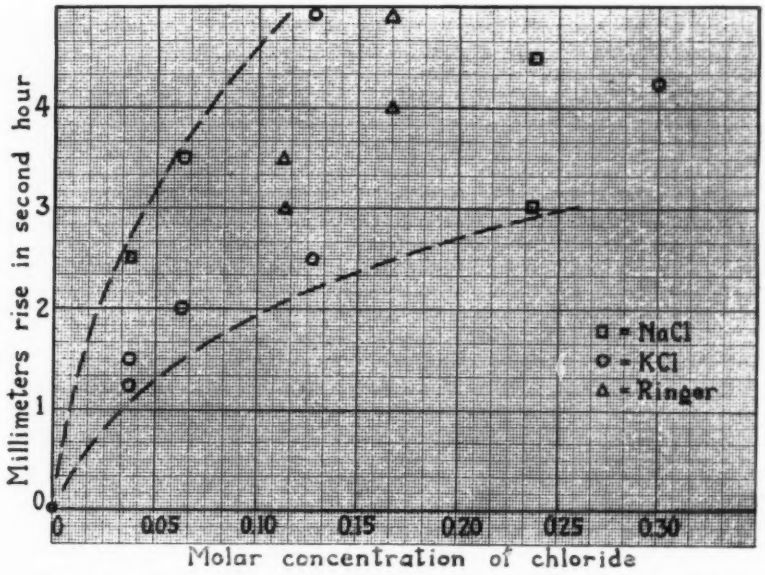

Fig. 3. Comparative rates of passage of water through inverted frog skin (from inner surface to outside surface), from pure water into salt solutions of various concentrations. The correlation with concentration is within the approximate limits outlined. The rate of passage is similar for sodium and potassium salts.

The "permeability" of frog skin varies, like that of a non-living membrane, according to the conditions of each observation. The chief condition is, again, the chemical environment of the membrane. This influence is manifested in comparing the rates of passage of water, which, it may be seen in table 1, vary in accordance with the solutes present on both sides of the skin. The factors found to be of importance are the concentration of the solutes, as shown in figure 3; and the ionic nature of the solutes. There is some evidence that the electrolytes further differ among themselves according to the valence of certain of their ions; and according to the reaction or hydrogen-ion concentration.

The passage of water through frog skin also varies sometimes in accordance with its previous treatment. This is most evident in figure 4, where a previous treatment with M/1 sucrose caused water to pass freely through the skin into M/20 sucrose, while previous treatment with Ringer's solution, or fresh isolation from the frog, caused water not to pass into M/20 sucrose solution.

The passage of water also varies with the morphological orientation of the skin. The latter variable has been frequently recognized (9), (10),

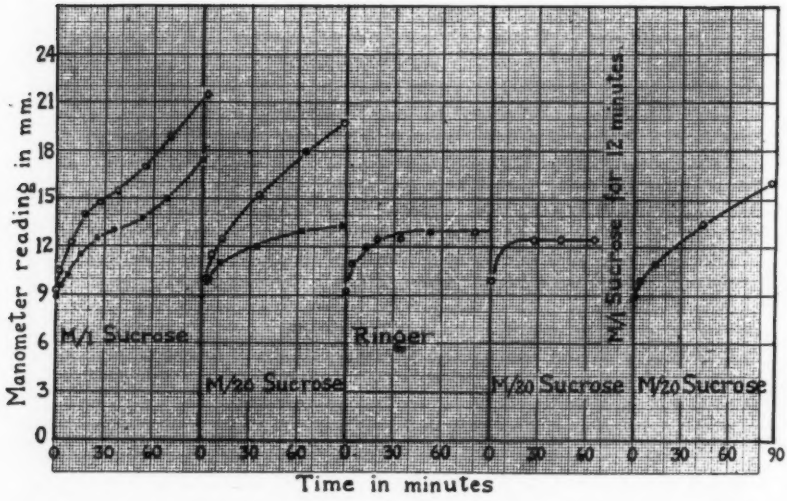

Fig. 4. Time course of the passage of water through two pieces of frog skin into successive solutions. Open circles, skin with inner surface bathed in tap-water; dots, skin with outer surface in tapwater. It is apparent that after treatment of the outer surface with strong sucrose solution, the passage of water into weak sucrose solution is rapid; after treatment with Ringer G water no longer passes into the weak sucrose solution. The effect is reversible. The strong sucrose treatment of the inner surface has no such effect.

and its mechanism will be discussed below. The morphological influence in general exhibits itself most strikingly (table 1) when the inside of the skin is bathed in pure water. Under such conditions water is transported through the membrane into almost any solution which bathes its outside, though not into pure water. When the outside of the skin is bathed in pure water, as it normally is, then almost no water passes. An exception to this occurs when the inside of the skin has been bathed with a strong solution of a non-electrolyte, such as M/1 sucrose.

Hydrostatic pressure has a particularly potent influence upon the initial

diffusion of water through both artificial and living membranes. In the experiments with 50 cc. collodion sacks (1) it was found that a hydrostatic pressure of one-fiftieth atmosphere might prevent water from diffusing into a solution exerting an osmotic pressure of twenty atmospheres, or a solution exerting an electrostatic attraction for water of forty atmospheres. With isolated frog skin similar anomalies occur. Apparently mechanical pressure causes a "slippage" of some sort in the system.

The treatment of collodion membranes with gelatin or other water-soluble protein markedly changes the properties of the membrane in regard to diffusion, as Loeb (11) demonstrated. Throughout the present comparative study of membranes we have used protein-treated membranes, since all living membranes contain proteins. Isolated frog skin, which has been killed by long standing or by chemically toxic agents, allows water to pass through it into various solutions in accordance with the rules for protein-treated collodion membranes (11). When completely dead, frog skin no longer shows marked morphological orientation.

III. Factors in the behavior of isolated frog skin. The diffusion of water through all membranes is greatly influenced by substances dissolved in the water. These influences upon non-living membranes are known to be of two kinds, osmotic or concentrational, and capillary or electrostatic. Non-electrolytes exert only the former, electrolytes exert both influences. Electrostatic influences are obvious only in dilute solutions, while osmotic are obvious only in concentrated solutions.

The rate of diffusion of water in response to *osmotic* forces is related to the osmotic pressures of the water-attracting solutions. This relation is expressed by the so-called "law" of Fick (12), which describes quantitatively the movement of water as well as that of solute. In the present study our attention has been drawn chiefly toward electrostatic influences upon diffusion, for if the passage of water through living membranes is a diffusion, it is very often not due to osmotic forces.

To illustrate the fact that the two sets of forces in diffusion, viz., the concentration or osmotic force, and the electrical or ionic force, are both surface phenomena and not volume phenomena, a pair of diffusion systems with collodion membranes was set up, one consisting of a very small membrane, the other of a large one. The small membrane was of collodion of the same composition as in the sack, and was tightly cemented with more collodion to a short glass tube, as described above. Its diffusion surface was 2.5 square centimeters, while the volume of liquid contained inside the tube was 11.0 cc. The large membrane was a 50 cc. sack which had a surface of 60 square centimeters and held a volume of 48.0 cc. Both were coated inside with gelatin. Each dialyser, closed at the top with a stopper containing a 3 mm. manometer tube, was filled with M/32 Na_2SO_4, and was immersed in distilled water.

The initial diffusion rates were approximately as 2.4 is to 50, or 1 to 21; and the maximum rise in the first system was obtained in 30 hours, while in the second, the same maximum was reached in 1.5 hours. When plotted to different scales which were in time as 1:20, the curves were approximately superimposed. Now, the ratio of the surfaces was as 1:24, while the volumes were as 1:4.4. Thus it is evident that electrostatic diffusion proceeds at a rate proportional to the surface involved, when other things are equal. The maximum height attained in the two manometers compared above was also alike, 135 mm. in one and 145 mm. in the other. This latter equality, however, is entirely due to the presence of like concentrations in the systems, and has nothing to do with the volumes concerned within certain limits.

To compare the rates of diffusion through equal areas of gelatin-treated collodion membrane and of frog skin, we used equivalent conditions throughout. With regard to electrostatic diffusion we attempted to use solutions of trivalent electrolytes; but these are all toxic to frog skin in any considerable concentration. The only comparison we have is therefore with dead or dying frog skin. A 0.01 M solution of ferric chloride served to attract water through frog skin in either orientation, but somewhat less rapidly, both initially and during 24 hours, than through artificial membranes. This retardation is probably due to the inability of the ferric chloride to penetrate into the skin to a great extent, being precipitated either as ferric hydroxide or as an organic salt. A 0.01 M solution of potassium ferricyanide failed to attract water through dead frog skin; this may be due either to an alkaline "reversal point" of the skin, or to chemical combination between the ions and the skin.

The above question can however be answered with regard to osmotic diffusion. A M/1 solution of sucrose attracted water with equal intensity with frog skin in either orientation, dead frog skin, or collodion.

We saw above that a M/1 solution of sucrose changed the behavior of frog skin toward the passage of water, making it "permeable" to it, and so it is not surprising to find that M/2 NaCl does not attract water osmotically through frog skin, while it does through collodion membranes. In other words, as long as the morphological interior surface of the skin is or recently has been bathed by univalent electrolytes, it does not allow water to pass inward through it.

But in the same circumstances water may pass outward through the skin. If fresh frog skin is placed with a hypotonic solution inside and water outside, water initially goes outward through the skin. This is especially shown by KCl solutions, as seen in figure 2 (curve A_1); but holds also for other solutions, including glycerine. This "negative diffusion" occurs only initially, and after two hours at most it ceases, even when the hydrostatic relations are reversed. Also, if the same skin is

again set up in the same hypotonic KCl (as in curve A_2, fig. 2), or in hypotonic NaCl, the "negative diffusion" is no longer exhibited. It is evidently an effect of the previous contact with lymph, and the original behavior can be restored by treatment with Ringer's solution.

As long as Ringer's solution bathes the inner surface of a frog's skin, but little water exchange occurs. This is brought out clearly by table 1. This is a fair substitute for the environment which preserves the regulating ability of the skin upon the living animal. It may be thought that frog skin habitually works to maintain a particular concentration difference between inside and outside. That this is not true is indicated by the fact that with the same solution on both sides there may be no water exchange. Thus (table 1), with water on both sides there is no passage; with Ringer's solution or Locke's solution on both sides there is no passage of water. It is a striking coincidence that artificial membranes behave similarly, for when they are bathed by two equivalent solutions, all diffusion potentials are nullified.

When three frog skins were oriented alike and piled together, water passed through them (into 0.24 M NaCl) at the same rate as through one. When to these three was added another layer of skin, oriented in the reverse way, water still passed, and only slightly more slowly than before. From this experiment it must be evident that frog skin is not a valve-like system; that intensities of some sort are at work.

Dead frog skin behaves in general like a gelatin-coated collodion membrane toward the passage of water. Both osmotic and electrostatic influences of the solution are then exerted predictably. Initially dead skin shows differences of morphological orientation, as has often been described (9), (10), (13); but we find that this influence of orientation upon diffusion is no greater than the irregular initial influences of the first hour of water passage through living skin. The dead skin therefore influences morphologically only the initial solute exchanges and the like.

The above are the facts concerning the passage of water through surviving frog skin as we have observed them. Most of these facts are new, for other investigators (9), (10), (13) of water diffusion through isolated frog skin did not have a knowledge of the immense susceptibility of living tissues to electrolytes and non-electrolytes. Matteucci and Cima (9) used very high concentrations of non-electrolytes, and Reid (13) used high hydrostatic pressures, either of which, we have shown above, leaves little trace of the normal function of frog skin.

IV. Experiments with intact frogs. To describe the behavior of frog skin in its normal situation toward the passage of water, we have studied volume changes in whole living frogs. Animals were immersed in various solutions and their body weight changes were measured. It was long supposed that the kidneys were the regulators of osmotic relations

in frogs. That the kidneys play a minor rôle, or none at all, at least in initial adjustments of frogs to changed conditions, has been, we believe, disposed of by the experiments of Przylecki (14).

For the present purpose we are concerned only with the initial fluid exchanges. It was found that the initial period of exchanges when frogs are placed in dilute solutions, occupies 6 or 7 hours at room temperature. This is also borne out by the classical data of Durig (15). We give, in

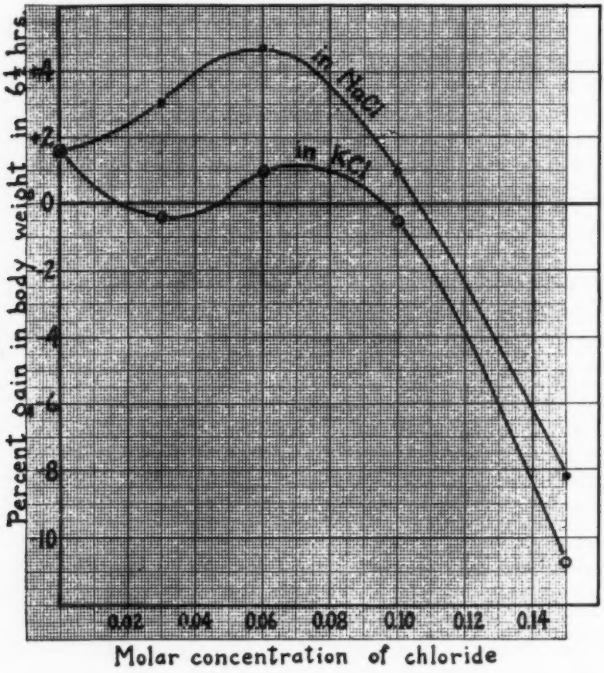

Fig. 5. Passage of water into living frogs while immersed in various concentrations of NaCl or KCl for 6½ hours, which in most cases was the time of maximum body weight changes. Each point represents the average gain in body weight for 3 frogs.

figure 5, the average amounts of body weight change in frogs when placed in solutions of various concentrations. It will be seen that not only the concentration makes a significant difference, but the kind of salt which now bathes the outer surface of the skin is significant when in concentrations less than those isotonic with frog lymph.

As Durig (15) pointed out, there is an obvious relation of the outer osmotic pressure to the osmotic pressure of the frog's body fluid. There is no body weight change at some concentration of the outer fluid which is

equivalent to about 0.11 M NaCl, where the outer fluid is isotonic or more probably slightly hypotonic to the inner one. In higher concentrations the passage of water is predominantly osmotic; below this concentration it is *not* osmotic. If we are to understand what frog skin regularly does, we must realize that it is maintaining a certain inequality of concentration between inner and outer solutions.

The relation of the experiments with isolated frog skin to those with living frogs is obviously a matter of chemical environment. Blood supply and nerve supply seem to make little difference, since the same results are given in the two cases in the same chemical situation. With the living frog skin bathed on the inner surface by a salt solution which is fairly similar to the composition of frog lymph, and bathed on the outer surface by pure water, water does not penetrate (fig. 1). In this situation there is just as much a fluid equilibrium as there is throughout the normal frog's life. But when the outer surface is in a low concentration of NaCl, water slowly penetrates the frog's skin, whether intact or isolated.

It is purely a matter of definition whether we are to regard the failure of water to pass as a "reversible impermeability" of the membrane, or a case of *improper circumstances* as supplied by the medium in conjunction with the membrane. On the one view "impermeability" changes to "permeability to water" when certain electrolytes are added to the outer medium. On the other view the electrolytes *in the solution*, together with other factors, control the passage of water. In either case it is evident from our data that effects of previous media tend to persist, and that there are many degrees of "permeability," or of "circumstance."

In the case of frog skin, and in other fresh-water animals, it has for a long time been thought possible that no energy is expended ordinarily to prevent the diffusion of water; that we have a valve mechanism. But in the case of other tissues, which function similarly, such as the intestinal mucosa and the kidneys, it is well known that energy is expended to oppose or to add to diffusion forces. A review of the numerous experiments on absorption (16) shows that many of them were experiments on physical diffusion, for we can now define the conditions for diffusion experiments and distinguish them from the normal conditions for absorption or secretion experiments.

In the case of the frog skin the practical conditions for preserving its "one way permeability" are the presence of nearly pure water on the outside, and of some sort of an electrolyte solution inside, preferably one which simulates frog lymph in composition. In the case of the intestinal mucosa, absorption can be studied only as long as a normal circulation, temperature, and environment are preserved on the inside of the tissue. Analogous indispensable conditions will be found for the study of the normal permeability of every living tissue. For most of them it is evi-

dently true that only under sufficiently unusual conditions will the living tissue act physically as a diffusion surface. Only then do such membranes behave like protein-coated collodion membranes, and are under the control of the composition of the solutions which tend to pass through them in accordance with the quantitative relationships of salt-electrostatic diffusion (7), (17).

It can be shown that the diffusion of water through two thicknesses of collodion membranes occurs just as rapidly as through one of them. From this it is not surprising that diffusion through dead frog skin was found to be just as rapid as through a collodion membrane. It has been known from the time of Graham (18) that diffusion of solutes through a column of agar gel or the like is just as rapid as through a column of water of the same dimensions. We may infer that a membrane, as long as it is mechanically pored, and under the proper surroundings, does not slow the diffusion of water no matter how thick it is when the permeability, the conditions, and the membrane are uniform; diffusion is always at a rate proportional to the amount of diffusion surface, and this we found to be the case.

V. The relation between the passage of water and electrical potential. Is it possible to construct a picture of the intimate relationships in frog skin membranes that shall account for the phenomena described? That frog skin is polarized in other respects than in relation to the passage of water through it has long been known. From consideration of the function of the normal skin it is obvious that the passage of all diffusible solutes must be similarly irreciprocal; this has often been noted, and most recently by Jacobs (19), Wertheimer (10) and Przylecki (14). Seventy years ago DuBois Reymond (4) found that frog skin exhibits an electrical potential difference between its two sides, and this was the starting point for extensive study of electric currents in living tissues. Many of these studies (20), (21), (22), (23) have been on frog skin itself, and by investigators generally it has been found that the potential is varied chiefly by changes in the electrolyte composition of the solutions which bathe the skin.

Having found that the passage of water is controlled chiefly by the chemical media, it is natural to compare the effects of various solutes upon the two phenomena. For this purpose we may utilize the most recent and most exhaustive data of Hashida (23). Hashida studied the electromotive forces of frog skin when connected to an electrometer by Ringer-calomel electrodes. In all his experiments Ringer's solution bathed the inner surface of the skin, while the solution bathing its outer surface varied. He always corrected for the diffusion potential between the Ringer's solution of the half cell and the outer solution, so that he believed the chief remaining potential was between outer solution and skin. The possibility of a change in the potential between the inner Ringer's solution and the frog

skin, he considered to be relatively small. Hashida found that both the concentrations and the composition of the outer solution varied the P. D. across the skin. Unfortunately he studied concentrations only up to 0.12 M; but in practically every case the P.D. was a maximum slightly below 0.12 M, and then fell to about zero with infinite dilution. He demonstrated that it was chiefly the cations and in particular sodium, which varied the P.D. at any given concentration; anions whether univalent or bivalent had less effect.

These same effects of concentration and of cations hold qualitatively for the passage of water through frog skin, in toto or isolated, when Ringer's solution bathes its inner surface (fig. 5). The passage of water is thus parallel to the P.D. of the isolated skin, whether upon the normal animal or similarly isolated.

Hashida found that the outer surface of the frog skin was always negative compared to the inner surface in 0.12 M solutions. Since water travels only from the inner toward the outer, it is evident that water is going toward the negative area. We already know that this is exactly the direction water always takes in electrostatic diffusion, as Loeb (24) has adequately shown. Under most conditions we know that water bears a positive electrostatic charge compared to other bodies, and it is possible so to picture the migration of water through the frog skin. The migration of water toward negativity must hold so long as in the presence of univalent and bivalent cations the solutions remain on the alkaline side of the "reversal" point of the proteins in the membrane, whether gelatin-collodion or frog skin. There is no indication that in frog skin there is any exception to this rule concerning the direction of the passage of water.

The qualitative correlation between electrical potential and diffusion allows us to ask whether one is the cause of the other. We know from the work of Quincke (25) and Freundlich (26) that water migration is not proportional to the measurable electromotive forces, as Girard (27) and Loeb (24) imply; yet a measurable P.D. must be present in the same direction as the electrostatic flow which occurs. As far as we can discern, it is perfectly permissible to suppose that with lymph on one side of the skin and water on the other side, the electrostatic potential maintained and the electric current expended by the skin are *just sufficient* to oppose the water diffusion which would ordinarily occur from pure water into lymph.

For the present, the diffusion of water through frog skin can hardly be evaluated in terms of electrostatic potentials. There are in the latter several factors incapable of direct measurement, as partially indicated in the formula for rate of electrostatic diffusion given by Smoluchowski (17). At least one of these factors, the potential between the pore walls and the water in the pores, will probably never be directly measurable.

Hardy (28) pointed out in 1911 that electrification of a surface as com-

pared to an adjacent medium must modify the passage of substances into and out of a living cell. He, and others since then, have shown that electrostatic charges exist between most living units and their medium.

Easier than to demonstrate conclusively that one-way permeability to water is correlated with electrostatic conditions in the tissue, is to show that no other form of energy expenditure known will maintain a water balance such as frog skin does. We were driven to this conclusion when we attempted to use the method of Van't Hoff (29) for calculating the work done in separating two solutions of unequal concentration. The method has been applied to the kidney's work of concentrating its excretions, by Dreser (30) and others. It turned out that a separation of solutes from pure water required that infinite work be done, and this convinced us that no machine, living or dead, could maintain a body fluid against exterior pure water *by osmotic means.*

Frog skin normally, therefore, maintains an unequal distribution of water along with and possibly by means of its maintenance of an unequal distribution of electrostatic charges. Does the qualitative correlation of water flow with P.D. hold in other than the normal media? Immersion in other solutions than Ringer inside and water outside, alters the functional capacity of the skin in at least two ways; *a*, it changes the P.D. across the skin; and *b*, it changes the relative concentrations which tend to cause osmotic (and perhaps also salt-electrostatic) diffusions. The latter can be kept immeasurably small by studying dilute solutions, for we found with collodion membranes that solutions which contained chiefly univalent salts in concentrations less than 0.1 M had no measurable influence, either osmotic or electrostatic. Then we may be sure that any effect found in dilute solutions, or any difference found between dilute solutions of equal concentrations of equivalent salts, is due to a change in the skin.

Though our own measurements of P.D. across frog skin are not adequate as yet, the data contained in the papers of Galeotti (20) and Orbeli (21) correlate throughout with the rules for water diffusion; they found that with the same concentration of potassium halide on both sides of the P.D. was always zero; while the highest values were with sodium salts. Thus the connection between the two phenomena seems to hold in abnormal circumstances. And finally, we know that dead frog skin gives a slight P.D. in the reverse direction from the living, and we have found that the diffusion of water is then initially slightly reversed; as Wertheimer (10) also found, though by a gravimetric method which we have found to be very uncertain.

It seems natural to ask whether the passage of salts through frog skin is also correlated with its electrical potential. We know from the living animal that in general all salts tend to pass in the opposite direction from water. Upon isolated skin few studies have been made, but it appears

from Wertheimer's (10) qualitative data that, in the cases known, the passage of salts is from negativity to positivity through the skin, the direction being reversed under conditions where the P.D. is reversed. It seems impossible to correlate selective salt absorption with electrical phenomena; but it has recently been demonstrated by Bethe (39) that significant electrostatic influences upon solute diffusion exist. That there is, besides, a relation between the total or osmotic concentration of salts and the electrical polarity seems inevitable.

The question next arises as to whether we have any information as to the mechanism of maintenance of electrical potential. In a previous paper (1) we showed that an unequal distribution of the salts characteristic of living tissues could not *of itself*, by mechanisms known at the present day, furnish sufficient potential to bring about marked diffusion of water. The fact brought out above, that sodium ions are chiefly responsible for the maintenance of a potential, indicates that the salt actions are not primarily related to valence, such as is most obvious in studies of electrostatic diffusion.

Our observations indicate that the passage of water through frog skin becomes very slow in several cases where the same solution is on both sides of it. This may be taken to indicate that differences in the distribution of ions are necessary for the maintenance of the electrical potentials, as they also are for ordinary salt-electrostatic diffusion.

It has long been customary to explain "bioelectric currents" as due to the unequal distribution of electrolytes in living tissues. This view has been thoroughly discussed by Lillie (31). There is, no doubt, a correlation between the distribution of ions and the distribution of electrical potentials. But it is a consequence of our hypothesis that either the mechanism of unequal water distribution is distinct from the unequal electrolyte distribution, or the electrostatic potential is set up and maintained by some other means than by the distribution of electrolytes. In either case it must be emphasized again that the measurable potential of a concentration cell is very different from the potential of a membrane pore (26).

VI. The relation between permeability and diffusion. If permeability is inseparably connected with electrostatic potentials, it is obvious that we have arrived at an explanation or generalized description of permeability to water. Moreover, we are now able to view permeability in non-living and in living membranes as one and the same thing. It has gradually been realized that the permeability of non-living membranes, such as those used in measurements of osmotic pressure, is not a mechanical matter of sieve-like action. As far as we are able to learn, the electrostatic description of a membrane, such as that of Tinker (8), sums up adequately what is known of its physical behavior. Passage or non-passage of solute particles, and presumably of solvent too, depend upon the attractions and

repulsions of the charged diffusible substances and the charged membrane pores, though some membranes may act like sieves for very large molecules such as proteins. The relative potentials between solid pore wall, solvent particles and solute particles, together with the current passing along the pore, determines not only the rate of diffusion, but osmotic pressure, and permeability. If this be true, the study of permeability is the study of diffusion and *vice versa*.

The difference between a living membrane and a non-living one lies then in the fact that a living one often has mechanisms for maintaining and for varying the potential residing in its surfaces. In such a mechanism energy is continually expended to build up an electrostatic potential, while diffusion of matter and of energy continually lowers it. It is now manifestly impossible to speak of permeability in most living systems as a passive process, as indeed the inadequacy of all the current theories of permeability shows. Ordinary chemical equilibrium is obviously continually prevented, by the energy transformations involved. For example, one can be rather certain that energy is used up in the transfer of water through frog skin, when it occurs under any conditions where it will not occur through a protein-coated collodion membrane. This is especially apparent when transfer occurs from 0.06 M KCl into pure water, or the like.

It is fair to conclude that permeability to water is not different from the *conditions* for water diffusion. Permeability only depends in small part upon the particular structure of a phase boundary; it depends, at least up to its limits of variability, upon electrical conditions. Ready penetration is, therefore, rapid diffusion, but it corresponds also to "high permeability." They are inseparable, and not due to independently changing structural qualities. Moreover, the behavior of frog skin is a striking illustration of the incorrectness of speaking of a *general* permeability condition; for the permeabilities to water, sodium, sucrose and electricity are fairly independent of each other.

There is an interesting relationship between potential differences across frog skin, and the antagonistic action of salts upon tissues generally. In all the cases which have been analysed it has turned out that salt antagonism is almost exclusively an interaction of cations. Usually such a relationship is intelligibly due to the employment of experimental media which are on the acid side of the tissue's "reversal point." Now Hashida (23) has shown that the P.D. in frog skin, too, is chiefly influenced by cations. He, and Galeotti (21) also, found that among the univalent cations sodium plays a predominant rôle in the maintenance of electrical potentials, while potassium and calcium are approximately neutral in their effects. But whenever the latter are combined with sodium, even in equal concentration, Hashida (32) found almost no reduction of the

potential due to sodium alone. It appears that sodium, by itself, is the chief controller of skin potentials. This gives a significant correlation with our finding no antagonistic influence of salts upon the water migration through the skin (fig. 3). And we found, as did Durig (15) and others, that when the normal frog is immersed in a potassium chloride or calcium chloride solution in concentrations less than 0.1 M, there is less intake of water by the frog than in a dilute sodium chloride solution.

It is well known that in the electrostatic diffusion of water through membranes, the amount of water transported is directly proportional to the electrical current expended (17). If the potential is constant, then, by Ohm's law the amount of current which passes will be a function of the electrical resistance. Electrical resistance in a living membrane is, upon our view, directly related to the penetration of water, and its use as a partial measure of permeability *to water*, is in so far justified (3), (33).

Now, at least, it must be apparent that permeability can be described only in quantitative terms. Qualitative studies of permeability have, however, apparently contributed one distinct point, namely, that on the whole only electrolytes are selectively dealt with as regards their transport through living membranes. One important exception to this rule is the case of d-dextrose, described by Hamburger (34). The rule, however, emphasizes the connection between the diffusing substances and the electrical phenomena. A more exact division is into polar and non-polar compounds; which has been elaborated by Jacobs (35). This division emphasizes the fact that all compounds are more or less electrolytic. Now, it seems that one can readily understand the important results of Overton (36) and of Brown (37) on this basis, for the compounds which are non-polar (usually non-ionized), they found to be very diffusible. In other words, it is not permeability, but impermeability, which needs to be explained in the case of the small distances concerned in cell-penetration studies. For, more polar solutes are capable of extensive selection, less polar ones are not, in any system involving either electrical potentials or ionic equilibria. When this point is properly substantiated, it would seem that there is little support for a solubility theory of permeability either in the form of the lipoid, fat, mosaic or emulsion modifications.

In a great many cases which have been studied, such as those by Brown (37) the same reagents, which themselves penetrated, produced penetration of water. It is gradually becoming established, it appears, that *changes* in permeability are not very selective, compared to set permeabilities. In numerous cases these changes are lasting, though reversible as in the effect of molar sucrose solution noted above (fig. 4).

Finally, it may be noted that the relationship between electrical potentials and selective passage of solvents and solutes, which has been used as the basis for a "theory of permeability" to water, has long been

regarded as probable. Its exact connection, however, could not be apparent until our general understanding of electrostatic diffusion of water had advanced as far as present status. For instance, Hermann (38), as a result of experiments on frog skin itself, regarded "bioelectric currents" as having much to do with processes of secretion and absorption. Many implications of the relation of electrical potentials to permeability phenomena in living systems are contained in the work of Lillie (31). The interpretation upon this basis of more extensive quantitative relationships in the processes usually recognized as secretion and absorption proper, will undoubtedly prove highly intricate. Though the protoplasts of plant cells and the various epithelia of the animal body exhibit many phenomena in common with frog skin, the extension of the present conception to them is not justified without direct study of each tissue.

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SUMMARY

1. The passage of water through frog skin was studied under normal conditions and in various unusual environments.

2. No direct relationship to concentration, nor to valency of dilute solutes, can be found in the passage of water through living frog skin, in comparison with the passage through protein-treated collodion membranes.

3. The morphological polarization of frog skin is preserved, in many unusual chemical environments, as long as it lives.

4. A conception of permeability to water, which is the only one known to apply to the case where pure water is separated reversibly from an electrolytic fluid, is based upon the electrostatic opposition to water passage by a variable electrostatic potential. The chief variations in potential are due to electrolytes in the medium, but the diffusion of water is probably not due directly to unequal distribution of ions.

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STUDIES IN THE PHYSIOLOGY OF VITAMINS

III. Quantitative Aspects of the Relation Between Vitamin B and Appetite in the Dog[1]

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Investigations of vitamin B phenomena have been for the most part qualitative in character. The syndrome characteristic of vitamin B deficiency has been quite clearly defined in most of the common laboratory animals and in man. Studies of the quantitative aspects of the subject have not been so extensive. Aside from comparisons of various body tissues and common foods with respect to their vitamin B content (Osborne and Mendel, 1917; Cooper, 1912; McCollum and Davis, 1915; McCollum and Simmonds, 1918), and such studies as Steenbock and his collaborators (1923) and Drummond (1918) have made of the behavior of rats on diets containing varying amounts of the dietary essential, few experiments of a definitely quantitative character have been performed. The outstanding paper dealing with this phase of the problem is perhaps that of Osborne and Mendel (1922), who showed quite clearly that the amount of dried brewery yeast necessary to supply the water-soluble accessory factor to rats on a diet otherwise free from it, bears a fairly definite relation to the size of the animal as expressed by the body weight. Any extension of such a line of investigation possesses value, when it is realized that such data contribute to a solution of the question as to the factors that determine what the vitamin B requirement of an organism shall be, and the real physiological rôle of this dietary essential. Until such knowledge is obtained, any estimation of the amount of vitamin B required by the normal individual and in therapy cannot be made in accordance with any line of reasoning having a scientific basis.

Most of the vitamin B studies described in the recent literature have been carried out on the rat or the pigeon. It is obvious that the determination of all of the factors underlying vitamin B requirement may

[1] A preliminary report of these experiments was presented to the American Society of Biochemists at St. Louis, Mo., December 27, 1923. The expenses of this investigation were defrayed in part by a contribution from the Russell H. Chittenden Fund for Research in Physiological Chemistry.

be very difficult, if the investigations are confined to one species. We believe, therefore, that the experiments to be described in this paper possess additional significance in that they were performed on another species of animal, namely, the dog.

In our earlier tests of various products containing vitamin B for their ability to restore the appetite to dogs subsisting on diets relatively free from this accessory food factor (Cowgill, 1921) it was learned that the relative potencies of the substances examined in this way are much the same as the relative contents of vitamin B when determined by the rat or the pigeon method. As stated elsewhere "this parallelism indicates that the physiological effects of these products are due to a common factor, probably vitamin B." Table 1 is a summary of the earlier tests made by Karr (1920) and one of us (Cowgill, 1921) and sets forth the relationship just stated.

TABLE 1

Vitamin B and appetite in the dog

SUBSTANCE TESTED	VITAMIN B CONTENT (RAT AND PIGEON METHODS)	APPETITE-PROMOTING POWER (DOG METHOD)
Yeast, brewery (tested by Karr*)	+++	+++
Yeast, bakers' (tested by Karr*)	+	+
Tomato, suspension of (tested by Karr*)	++	++
Rice polishings, alcoholic extract of	+++	+++
Wheat embryo, alcoholic extract of	+++	+++
Navy bean, alcoholic extract of	+	+
Yeast Vitamin Powder (Harris)†	++++++	++++++
Vitavose‡	+++	+++
Liebig's beef extract, commercial	−	−

* W. G. Karr: Journ. Biol. Chem., 1920, xliv, 255.

† From the Harris Laboratories, Tuckahoe, New York.

‡ From the Ward Baking Company, New York.

It is not intended in table 1 to convey the idea that an *absolute* correlation exists: that, for example, the brewery yeast, alcoholic extracts of rice polishings and wheat embryo, and the "Vitavose," with their "3 plus" signs are of exactly equal vitamin content and appetite-promoting power. So far as the tests showed, these products seemed to be of approximately equal value, each being much better than the bakers' yeast, somewhat better than the tomato suspension, and certainly much less potent than the yeast vitamine powder.

This parallelism suggested an approach to the problem of ascertaining the dog's vitamin B requirement using the restoration and maintenance of the urge to eat as the criterion for the satisfaction of the vitamin need. The availability in quantity of yeast vitamine powder (Harris)[2] and Vita-

[2] From the Harris Laboratories, Tuckahoe, New York.

vose[3] solved the problem of having suitable amounts of materials relatively rich in vitamin B and obtained from two widely different sources, namely, yeast and wheat embryo. As a control substance for such experiments commercial beef extract was used; this material does not contain vitamin B (Damon, 1922).

The Harris product is made following the essential details of the directions given by Osborne and Wakeman (1919) for preparing their yeast fraction which is rich in vitamin B. This fraction as prepared by these investigators represents a concentration of the yeast vitamin of about ten times. The commercial product as prepared by Doctor Harris appears to be about seven times as potent as good brewery yeast. Vitavose is

TABLE 2

Diet IV

	GRAMS	CALORIES*	PER CENT
Meat residue† (13 per cent N)	6.15	21.6	37.3
Meat residue† (10 per cent fat)		5.67	
Sucrose	5.63	23.5	34.1
Lard	3.00	28.4	18.2
Butter	1.12	9.46‡	6.7
Bone ash	0.4		2.5
Salt mixture§	0.2		1.2
Total	16.50	88.6	100.0

This kilo-unit contains 0.8 gram of protein B. One gram of this food mixture contains 5.68 calories.

* Loewy's factors are used, namely, 4.32 for protein, 4.18 for carbohydrate, and 9.46 for fat.

† From the Valentine Meat Juice Company, Richmond, Virginia.

‡ A 10 per cent correction for salt and water content.

§ Used by Karr (1920).

prepared from wheat embryo. Its chief ingredient appears to be maltose which has been produced presumably from starch by the germination process to which the wheat germ has been subjected. Judging from tests made on rats, this product is only slightly inferior in vitamin content to high grade brewery yeast.

EXPERIMENTAL. *Single administrations of vitamin-containing products.* Dogs were fed on artificial diets free from or very low in vitamin B in accordance with the method developed in this laboratory (Cowgill, 1923). The foods used were diets III and IV. The details of diet III have already been published (Cowgill, 1923), diet IV is shown in table 2.

[3] From the Ward Baking Company, New York City.

When the urge to eat had been partially or wholly lost, a single dose of the vitamin preparation was administered by stomach tube. The usual effect noted was a restoration of the desire for food over a period of days. The food intake as influenced by vitamin administration is shown graphi-

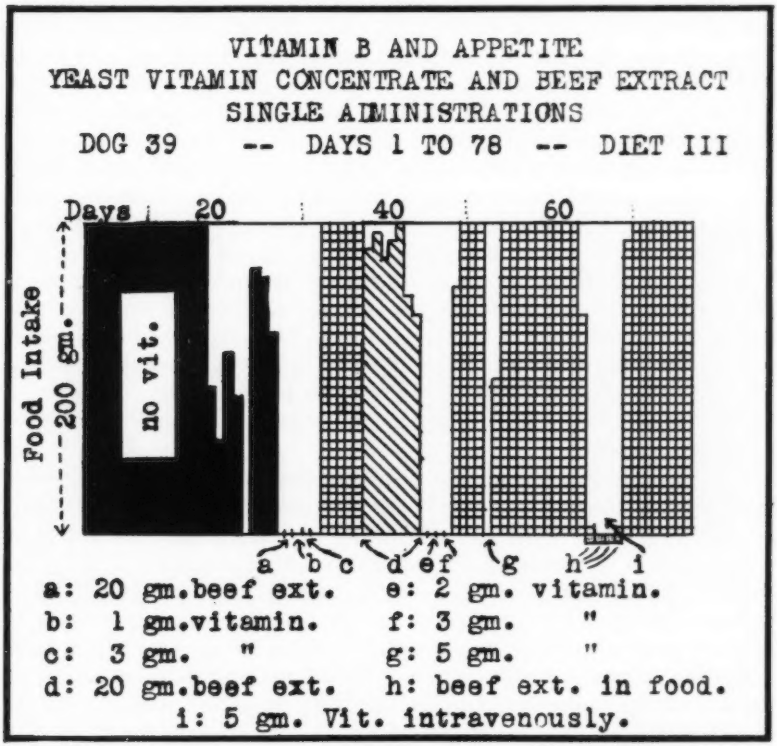

Fig. 1. The ineffectiveness of beef extract as an agent for promoting appetite is well illustrated by the experiments on dog 39. Neither the administration of large single doses of the commercial material, *a*, nor the mixing of it with the food, *h*, influenced the appetite favorably. The graded responses to vitamin administration as shown by the number of days over which the appetite was restored, are evident at *c*, *f* and *g*. The doses given at *b* and *e* were evidently subminimal. *Intravenous* injection of this vitamin preparation, *i*, was also effective in restoring the appetite.

cally in the illustrative charts presented. The ordinates represent the amount of food offered, which was usually planned to be such that, if entirely eaten, maintenance of body weight would ensue. Days are shown in the abscissae.

In figure 1, for example, it will be noticed that a perfect food intake

occurred for nineteen days. On the twentieth day slightly more than half the amount offered was eaten over a period of several hours during which the ration was left in the cage. After a period of irregular food intake shown by the black ordinate columns of different lengths, the food was refused entirely. At point *a*, a large amount—20 grams—of beef extract was given by means of a stomach tube. This did not restore the appetite. Two days later 1 gram of the yeast vitamin concentrate was given but without effect. In this case the experimental animal was a large one, and later experiments made it clear that this dose was subminimal. When 3 grams of the same preparation were given two days later, the appetite was restored promptly and a perfect food intake was maintained for five days. Loss of desire for food then appeared and administration of a large dose of beef extract was without effect. At *e*, the administration of 2 grams of the vitamin powder was ineffective whereas 3 grams again restored the appetite. At *g*, a 5 gram dose restored the desire to eat for ten days. At another time—*h*—when vitamin lack had affected the appetite, this dog could not be coaxed to eat the food when beef extract had been mixed with it, whereas 5 grams of the vitamin powder given intravenously produced the desired effect—*i*. This detailed description of figure 1 should enable the reader to understand without difficulty the other figures presented.

The results just described are typical of those obtained in our earlier trials and in themselves suggest many of the experiments which were subsequently carried out. Certain doses of vitamin product appeared very definitely to be subminimal; the administration of larger amounts to the same animal appeared to have graded effects in accordance with the amount of vitamin given. When a certain dose was tested on animals of different body weights, it was observed that the therapeutic values were different. Thus 3 grams of the yeast vitamin concentrate given to dog 44 (table 3) weighing 4.1 kilograms restored the appetite and maintained it over a period of 12 days, whereas the same dose given to dog 39 of 11.3 kilo body weight produced a similar effect over only 5 days. Calculations of the value of the product in milligrams of the powder per kilo of body weight per day, however, gave figures nearly the same, namely, 61 and 53 milligrams, respectively. In table 3 are presented the data from many trials of this character. Figures 1 and 2 illustrate this plan of experimentation.

The results of numerous experiments of this kind soon brought out at least one serious objection inherent in the plan itself. Animals differ with respect to the time required for the development of the symptoms of vitamin B deficiency. A young dog under the influence of the growth impulse tends to consume the deficient diet longer than an adult animal before revealing a loss of the desire to eat. This behavior is similar to

that characterizing young rats as described by McCollum (1909). This may perhaps be attributed in part to the more vigorous "hunger contractions" of the stomach in young animals (Carlson, 1916) and to the fact that a gradually developing gastric atony appears—certainly in dogs subsisting on such vitamin B-free diets as are described in this paper (Cowgill and Deuel, 1924). This atony develops more slowly in the young dog than in the old animal. Inasmuch as the symptoms characteristic of vitamin B deficiency seem to appear only when an organism metabolizes food in the absence of the accessory factor, the young animal's condition, when appetite is lost, is likely to be more serious than in the case of an old dog. Other factors besides age must play a rôle, however, because most of our animals were full-grown.

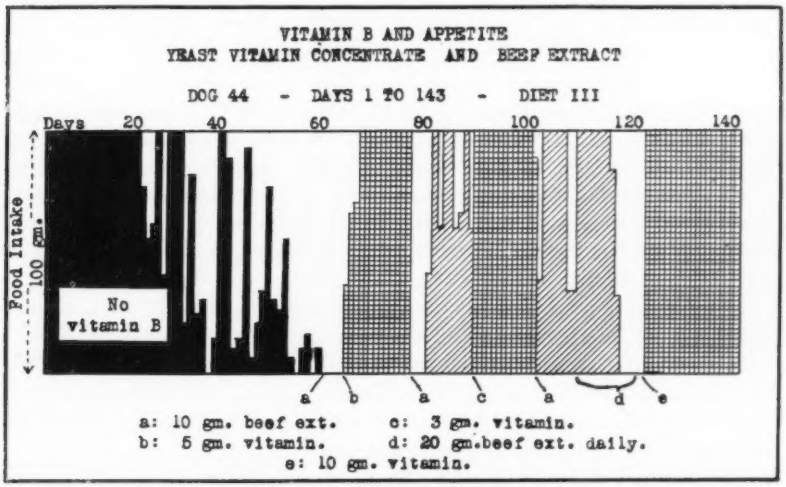

Fig. 2. The experiments with dog 44 show the graded effects on the appetite of administering various amounts of a yeast vitamin preparation, and the failure of beef extract to restore the urge to eat. Administration of the vitamin at *b* was made when the animal exhibited nervous and muscular symptoms of vitamin B deficiency. The beef extract treatment given on the 78th day—the second *a*—may be regarded as being ineffective, inasmuch as the partial restoration of appetite which occurred between the 80th and the 89th day does not differ essentially from that occurring between the 30th and 40th day. The positive effect on the appetite of the single dose of vitamin given at *c* is quite evident and in noticeable contrast to the previous "beef extract period." That large doses of beef extract given daily may have a *slightly* favorable influence on the appetite is shown at *d*, but such an effect is short lasting; this may possibly have been due to a very slight amount of vitamin B present in the material. The dog was small and the doses of beef extract relatively enormous. (See text and footnote 4 regarding evidence for the presence of vitamin B in beef extract.)

The question of the reserve supply of vitamin B stored in the tissues must also be considered. In view of the fact that there is as yet no good criterion by which to estimate the exact amount thus stored, this factor cannot be evaluated accurately.

In the light of these considerations, therefore, it is quite possible that not all the tests of the type just described were made on animals in conditions of vitamin B deficiency of equal severity. For example, reference to figures 1 and 2 will show that whereas dog 39 received no vitamin B over a period of thirty days, dog 44 went for sixty-three days before being given the dietary essential. In both cases vitamin was administered only after the appetite had failed completely for at least three successive days. The condition of dog 44 when receiving treatment was quite obviously more serious than that of the other animal. It showed such serious symptoms as clonic spasms and spastic paralysis on the sixty-sixth day when vitamin therapy was instituted, whereas dog 39 at the time it received vitamin exhibited merely an absolute refusal to eat the food offered. Quite obviously these two cases are not comparable. If an estimation of the number of grams of the vitamin powder per kilo of body weight per day be made similar to those listed in table 3 and based on the trial on dog 44 just cited, 0.090 gram is the result obtained as compared with 0.060 gram given by a second test on this same animal when its condition was obviously more comparable with that of dog 39, which gave 0.053 gram in two different trials.

In table 3 are presented the data from numerous tests of the kind just described, which appeared at all comparable.

In view of the considerations just discussed, it seems remarkable rather than otherwise, that so many trials should indicate the dog's vitamin B requirement in terms of the yeast concentrate to be between 50 and 60 milligrams per kilo of body weight per day.

The test on dog 44 which gave a result of 0.120 gram per kilo per day, is an interesting one (see table 3). In this case a very large dose of the vitamin powder, namely 10 grams, was given to a small animal weighing only 4.4 kilograms. The dog ate all the food offered for 19 days before showing any loss of appetite. Now if 0.060 gram be regarded as the amount required per kilo per day under these conditions, as indicated by the many tests summarized in table 3, the 10 gram dose should have been followed by a perfect appetite over a period of 38 days. As a matter of fact its effect lasted only half as long. How should this phenomenon be interpreted?

The observations of Van der Walle (1922) are suggestive in this connection. This investigator noticed that the urine of a dog on a vitamin B-free diet had no therapeutic value for polyneuritic pigeons, whereas the urine collected, when the animal was subsisting on a normal diet, was effective. Curatolo (1920) and Gaglio (1919) have also reported the

presence of vitamin B in urine. It has been shown by Cooper (1924) with respect to vitamin A that when a large dose is administered to an animal, various body fluids such as gastric juice and urine show an increase in content of this dietary essential. Is it not likely, therefore, that the tissues of dog 44 absorbed as much as possible of the vitamin B contained in the large dose administered, and dissipated the remainder through the various excretory channels? If this explanation is the correct one, then it appears that a dog's capacity to store this accessory factor is such as to enable it to subsist on a vitamin-free diet without symptoms of vitamin lack for approximately twenty days or three weeks.

TABLE 3

Single doses of vitamin B and appetite quantitative relationships

DOG	DAYS OF PERFECT APPETITE	AVERAGE OF BODY-WEIGHTS FOR PERIOD	KILO-DAYS	GRAMS GIVEN	GRAMS PER KILO PER DAY
	D	K	K × D	Vitamin B	$\frac{\text{Vitamin B}}{\text{K} \times \text{D}}$
		kgm.			
39	5	11.3	56.5	3 H	0.053
39	9	11.0	99.0	5 H	0.053
40	9	7.8	70.2	4 H	0.057
44	12	4.1	49.2	3 H	0.061
44	19	4.4	83.6	10 H	0.120*
55	11	7.2	79.2	5 H	0.063
56	5	6.6	33.0	2 H	0.060
54	4	5.9	23.6	2 H	0.084†
55	7	7.3	51.1	26 V	0.500
54	7	6.4	44.8	27 V	0.600

H, yeast vitamin powder (Harris).

V, vitavose.

* Does this mean that this dog could not store a great excess of vitamin B?

† This animal also required relatively more of the vitamin preparation than other animals when given it in daily doses.

In this connection it is interesting to recall that many feeding experiments are recorded in the literature in which diets now recognized to be deficient in vitamin B, were fed and loss of appetite on the part of the experimental animal supervened on or about the twentieth day. Steinitz (1898) fed dogs on artificial mixtures but after a fortnight or three weeks' vomiting occurred and the experiments had to be discontinued. The attempts of Forster (1873) to study salt metabolism were only partially successful because his diets on being rendered salt free by a process of extraction were thereby made free from vitamin B as well.

Perhaps the most striking instances of experimental difficulties of this sort are to be found in Abderhalden's experiments (1912) in which dogs

were fed diets containing amino acid mixtures instead of protein. Difficulty in making the animals eat the food was usually encountered on about the twenty-second day. In some of the more fortunate trials the dogs ate the mixture voluntarily for from seventy to eighty days and in those cases Abderhalden noticed that the animals *ate their feces*. Such results suggest that the intestinal excreta contain vitamin B, a suggestion abundantly confirmed by the behavior of some of our dogs. One of our animals (dog 58) fed on diet III showed a perfect food intake for over thirty days and never developed nervous or muscular symptoms characteristic of vitamin B deficiency. This behavior was unusual. This dog was a coprophagist. After having been placed in a metabolism cage from which the feces were removed whenever found, and allowed to subsist on the vitamin-free diet, this animal showed the usual appetite failure, which was corrected by administering the missing accessory factor. Similar observations on coprophagistic dogs might be cited in this connection. We believe, therefore, that the apparent success, which attended some of Abderhalden's experiments, may be explained in part at least by two facts: that the animals were coprophagists and that the feces contained appreciable amounts of vitamin B.

This belief receives support from the work of Steenbock, Sell and Nelson (1923) showing that rats having access to intestinal excreta thrive on a smaller vitamin B intake from the food than their companions who are prevented from eating feces.

Control experiments with beef extract. The evidence thus far presented in support of the contention that a relationship exists between vitamin B and the restoration and maintenance of the urge to eat, is essentially in accordance with the logical *canon of agreement*. A variety of products, which are chemical mixtures of different sorts, all have in common an appreciable content of vitamin B, and all show the physiological properties characteristic of this dietary essential. The proof of the relationship between vitamin B and appetite would be even more cogent if the *joint canon of agreement and difference* could be applied. Negative control experiments would satisfy this condition.

Beef extract has long been used as a savoring agent with both man and animals. In their discussion of suitable foods for metabolism animals, Caspari and Zuntz (1911) give particular attention to the problem of maintaining the appetite and suggest the use of meat extract, bouillon, etc., as aids in surmounting this difficulty. According to Pawlow (1902) the ingestion of beef extract in appreciable amounts results in an increased flow of gastric juice. Ivy and McIlvain (1923) have confirmed this by showing that beef extract placed in an intestinal loop promotes the flow of gastric secretion. It is conceivable that a substance commonly regarded as being capable of promoting the appetite and shown by experiment to

stimulate the flow of gastric juice may owe its value in dietetics to this fact. Inasmuch as Damon (1922) has shown that commercial beef extract contains no vitamin B,[4] this material appears specially suited for negative control experiments in such studies as are described in this paper.

Commercial beef extract was administered *per os* to dogs *A:* in single large doses *1*, when the animals showed the first sign of appetite failure[5] and *2*, when a loss of the urge to eat had been in evidence for some time;[6] *B:* in large daily doses over a considerable period administered *1*, apart from the food[7] and *2*, mixed with it.[8] *In every case, after a definite failure of the beef extract either to restore or to maintain the appetite had been demonstrated, vitamin B was given and a positive effect on the urge to eat was obtained.*

Illustrative experiments of this sort are presented graphically in figures 1 and 2; the results here given are abundantly confirmed by those obtained on four other animals.

Beef extract, when tested under these conditions, appears in some animals to have a slight effect in maintaining the desire for food, especially when the condition of vitamin deficiency is mild in degree. As the period of vitamin lack lengthens, however, the beef extract becomes ineffective; at such a time, however, the appetite can readily be restored by administering vitamin B. In other animals, beef extract administration has very little if any effect on the appetite in contrast to the remarkable efficacy of vitamin B therapy in this respect. It was very interesting to observe that with some animals, after daily doses of beef extract given apart from the diet had failed to maintain the appetite, the dog was persuaded once or twice to eat a minute amount—two to five grams—of the artificial food mixture after beef extract had been mixed with it. On succeeding days, however, even this mode of persuasion was ineffective. At such a time *vitamin B administration resulted in a prompt restoration of the desire to eat.*

It may be said, therefore, that control experiments with beef extract yielded negative results, thereby furnishing additional support for the belief that a definite relationship exists between vitamin B and the restoration and maintenance of the appetite. An additional conclusion that seems justified by the experiments with beef extract, is that the restoration of appetite resulting from vitamin B administration is *not* due to an increased flow of gastric juice. This has a bearing on the hypothesis of vitamin B function advanced by Uhlmann (1918) and others, that this vitamin acts to promote the flow of various digestive juices.

[4] Osborne and Mendel have shown muscle extract to contain *some* vitamin B; the amount, if any, present in the commercial material we administered could hardly have been significant. See Journ. Biol. Chem., 1917, xxxii, 309.

[5] For illustration see the second and third *a* in figure 2.

[6] *a* in figure 1 and the first *a* in figure 2.

[7] *d* in figure 2.

[8] *h* in figure 1.

Daily administration of vitamin B. In view of the objection that tests with single doses of vitamin-containing product are likely to be made on animals whose conditions of vitamin B deficiency are not the same and therefore not comparable, a second plan was followed. Definite daily doses of the preparations were given and the periods, over which the urge to eat could be successfully maintained, were made the criteria as to the satisfaction of the dog's requirement for vitamin B. With from 50 to 60 mgm. of the yeast vitamin concentrate per kilo per day indicated by the earlier experiments to be necessary, it was deemed advisable to experiment with doses of 20, 30, 40, 50 and 60 mgm. per kilo per day. The amounts of the yeast vitamin concentrate thus required by any animal in one day were such as could be placed conveniently in a no. 000 gelatin capsule. Each capsule was administered daily immediately before the food was offered. In the case of the "Vitavose," the daily doses experimented with were necessarily much larger. The daily dose of this material was dissolved in a small amount of water and almost invariably ingested by the animal voluntarily. Three charts illustrative of these long continued feeding experiments are presented.

Experiments with yeast vitamin concentrate. The behavior of dog 41 is illustrated in figure 3. A daily dose of 20 mgm. per kilo of the yeast vitamin concentrate was administered to this animal beginning at a time when it was recovering from severe nervous and muscular symptoms of vitamin B deficiency.[9] There was a pronounced spastic gait still evident indicating that the condition of vitamin deficiency had not been entirely cured. Inasmuch as the animal had lost considerable weight, an amount of food greater than that required for maintenance was being offered during the recovery period. For fourteen days during which the 20 mgm. per kilo per day dose was being given, not all of the food offered was eaten. The question arose as to whether the failure to eat all food offered was due to lack of vitamin B or to an adjustment of the animal to its energy requirement. Failure to make satisfactory gains in body weight during this recovery period indicated the former to be the real cause of failure to eat. The dose of vitamin product was therefore increased to 30 mgm. per kilo per day, using the body weight of this date as a basis for the calculation. The result for a time was quite satisfactory. By the 109th day, however, it had become quite evident that the energy content of the daily ration was too large and that this was exerting an influence on the desire to eat all that was being offered. Therefore a reduction in the amount of food to that estimated to be sufficient for maintenance at the newly established body-weight level was made. Calculation for this forty day period using the average body weight and the amount of vitamin product given showed

[9] Photographs of this animal before and after treatment by intravenous injection of vitamin B have been published. See G. R. Cowgill, THIS JOURNAL, 1923, lxvi, 164.

that 25 mgm. per kilo for 7.3 kilo was the amount actually administered each day. On the 109th day the dosage was adjusted to 30 mgm. per kilo per day using the new body weight as the basis for calculation. As will be noticed, the amount of food offered was still sufficient to permit a slight increase in body weight. Over the period of seventy-four days the actual dosage proved to be 29 mgm. for 8.15 kilo per day, a figure practically identical with the intended dosage of 30 mgm. per kilo per day. For fifty-eight days a perfect appetite was maintained. Then followed a two-weeks period of irregular food intake at the end of which symptoms of

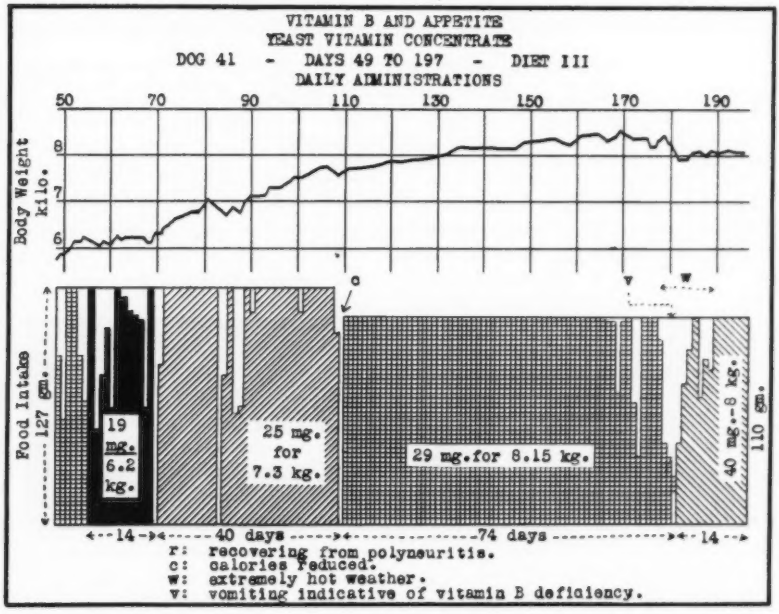

Fig. 3. For detailed description of these experiments with dog 41, see the text.

vitamin B deficiency such as foul breath and vomiting appeared. The daily dose of vitamin was then increased to 40 mgm. per kilo. In this case, the recovery of the desire to eat was not prompt owing doubtless to excessively hot weather which lasted for seven days; when it became somewhat cooler, the animal ate all the food offered. Unavoidable departures from the laboratory compelled us to terminate the experiment at this point. However, it had become quite clear that perfect maintenance of the urge to eat for as long as sixty days is *not* sufficient proof that the dog's requirement for vitamin B is being entirely satisfied.

With dog 44 the daily administration of 20 mgm. of yeast concentrate per kilo was concomitant with a perfect food intake for sixty days, but after the forty-sixth day a foul breath, usually a symptom of vitamin B deficiency, was noticeable. This animal developed pneumonia shortly after the urge to eat was lost, and died. This fact, however, in no way militates against this experiment being regarded as confirming the test with dog 41 in showing that 20 mgm. of the yeast vitamin concentrate per kilo per day were inadequate.

Figure 4 shows the results obtained with dog 45 using 20 and 30 mgm. per kilo per day doses of the yeast powder. Here it was quite evident that the 20 mgm. dose was not enough. The 30 mgm. dose proved to be

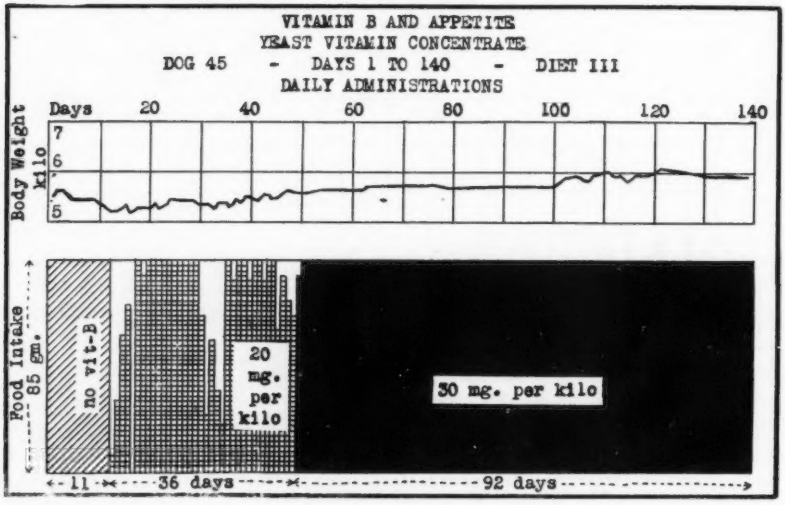

Fig. 4. The failure of 20 mgm. per kilo per day in contrast to a satisfactory result with 30 mgm. per kilo per day for dog 45 over three months is shown by this chart.

successful for 91 days. Unfortunately it was not possible to experiment longer with this animal.

In table 4 are presented the results obtained when doses of 40, 50 and 60 mgm. of the yeast concentrate per kilo per day were administered. With the exception of dog 53, which ate only about one-half the food offered on the fifty-third day—a condition explained as due to an adjustment of the animal to its energy requirement and promptly corrected by decreasing the number of Calories offered—*perfect food intake was maintained in all cases for as long as the experiments could be conducted.* Dogs 53 and 52 were successfully fed the artificial food mixture—diet III—plus 40 mgm. of yeast vitamin powder (Harris) for *four hundred and twenty,*

and *four hundred and seventy* days respectively, or approximately fourteen and fifteen months. They were successfully fed in the same way even longer, but became sick on moving to new laboratory quarters; this condition was corrected solely by administration of intestinal antiseptics[10] presumably free from vitamin B. If the interval during which these animals suffered from the intestinal trouble doubtless of bacterial origin be disregarded, these dogs subsisted on an artificial food mixture—diet III plus 40 mgm. per kilo per day of the yeast vitamin concentrate—for a total of four hundred and sixty-five and five hundred and fifteen days respectively. So far as we are aware, these are the longest successful recorded experiments in which artificial mixtures of foodstuffs have been

TABLE 4

Daily doses of yeast vitamin concentrate (Harris) and appetite

DOG	MILLIGRAMS PER KILO	DAYS INCLUSIVE	APPETITE (+ = PERFECT; − = IMPERFECT)	REMARKS
52	40	1–470	470+	Excellent condition throughout
53	40	1–52	52+	Received excess calories for growth
		53	1−	Decreased calories
		54–420	366+	Excellent condition
50	50	1–91	91+	Excellent condition throughout
51	50	1–91	91+	Excellent condition throughout
66	60	1–172	172+	Used in metabolism experiments
67	60	1–131	131+	Used in metabolism experiments

These data indicate that the minimum dose of yeast vitamin powder (Harris) required to maintain the appetite of the dog when fed under the conditions of these experiments is approximately 40 mgm. per kilo per day.

fed to dogs. That these conditions of feeding were satisfactory is further established by the normal autopsy and histological findings.[11]

Dogs 50 and 51 each received 50 mgm. of the yeast vitamin concentrate per kilo per day and showed a perfect appetite for as long as they were on the experiment, namely, 91 days. Dog 51 was an old animal that had

[10] Dog 52 was given a large dose of the yeast vitamin concentrate but this had no effect on the diarrheal condition. Both animals were then given tablets containing "sulphocarbolates of calcium, sodium and zinc, grs. 5, with bismuth subsalicylate, gr. 1–4 and aromatics" made by the Abbott Laboratories, Chicago, Ill. The dogs also received subcutaneous injections of "Milford's Anticanine Distemper Serum" and "Canine Distemper Serobacterin (Mixed)." This treatment proved to be effective in both cases.

[11] The authors are indebted to Dr. Isabel Wason of the Department of Pathology in Yale University Medical School for the detailed gross and microscopical examinations made on these two animals.

been in the laboratory for some time and had shown an erratic appetite, and lack of interest in the common laboratory fare of dog biscuit, etc. When given the yeast vitamin concentrate and offered the artificial food mixture, she showed at first only slight interest in the diet, although eating all during the course of three hours; after a few days she began eating all the food immediately when offered and continued behaving in this fashion for the rest of the experiment.

The 60 milligram dose was given to two animals (dogs 66 and 67) that were being used in metabolism experiments in which it was desired that they consume every bit of the artificial diet offered. This they did without fail for as long as the experiments lasted, namely, 172 and 131 days respectively—over four and five months.

These data indicate that the minimum dose of this yeast vitamin concentrate required to maintain the urge to eat in the dog fed under the conditions of these experiments is approximately 40 mgm. per kilo per day. It is possible that further experiments would have allowed us to be even more precise in this regard and to determine some figure between 30 and 40 mgm. as the correct minimum dose. However, our supply of the particular lot of "yeast vitamin powder" used in these experiments was insufficient to allow many more trials of this sort. Then too the indication of variability in the requirement among different animals, and a feeling that the method is not accurate enough to warrant more precise results led us to stop such experiments with dogs at this point.

Experiments with "Vitavose." Tests of this product on young rats[12] showed that it has a vitamin B content slightly less than that of brewery yeast, whereas the yeast vitamin powder (Harris) represents a concentration of brewery yeast vitamin of about seven times. With these relationships in mind, the preliminary tests using single administrations of Vitavose were made and the value of the substance per kilo per day estimated. These experiments were made some time after those with the yeast concentrate, when some experience with respect to the behavior of the experimental animals and some idea as to the degree of accuracy inherent in the method had been gained. As our supply of a single lot of the Vitavose was not large, only two preliminary tests were made. As shown in table 3, 500 and 600 mgm. per kilo per day were the results obtained. Tests involving the daily administration of the product were then carried out. Dog 55, after nineteen days during which 450 mgm. per kilo per day were given without perfect maintenance of the urge to eat, responded to a daily dose of 600 mgm. per kilo by showing a perfect food intake for 261 days—approximately eight and one-half months. The administration

[12] Dr. C. Hoffman of the Ward Baking Company kindly furnished us with data regarding the amount of Vitavose required to satisfy the vitamin B requirement of young rats.

of Vitavose was then discontinued. On the 368th day appeared a definite loss of the urge to eat. A lethargy probably associated with vitamin B deficiency had appeared previously on the 336th day. In this animal therefore, loss of appetite was not the first sign of vitamin lack. The tendency of this dog although manifestly unwell to partake of the vitamin-free food could only lead sooner or later to the manifestation of severe muscular and nervous symptoms characteristic of vitamin B deficiency. On the 405th day the animal was sprawled out, all limbs in extension with muscles vigorously contracted, and presenting a typical picture of a most severe type of the effects of this dietary deficiency. Repeated unsuccessful attempts were made to cure the condition by the administration of Vitavose through a stomach tube, but the animal was unable to retain the substance in the stomach. In this experiment, therefore, the urge to eat was maintained successfully by daily measured doses of Vitavose for eight and one-half months *and upon merely withholding the vitamin preparation while keeping all the other dietary factors unchanged, the development of the typical picture of vitamin B deficiency resulted.* Quite apart from the quantitative aspects of the subject this would seem to be a perfect demonstration of the vitamin hypothesis.

The result with dog 56—figure 5—confirms this demonstration. With this animal, the administration of the same amount of Vitavose—600 mgm.—per kilo per day was followed, after a latent period of 3 days, by perfect food intake for 288 days or approximately seven and one-half months. The daily dose of Vitavose was then discontinued. A week later, loss of the urge to eat occurred. After 24 days of irregular food intake associated with a gradual decline in body weight, a single dose of 37.5 grams of Vitavose was given. The restoration of appetite was prompt and lasted over eight days. Three days later, when all food had been refused, 18 grams of Vitavose were given, again followed by a prompt return of the desire to eat the food offered. Seven days later the experiment was terminated.

On the basis of the above results, it appears that the minimum amount of wheat embryo vitamin (Vitavose) required to maintain the urge to eat in the dog under the conditions of these experiments is approximately 0.6 gram per kilo per day.

Discussion. A comparison of these two vitamin-containing preparations based on the results described above gives the following ratio of potencies:

$$\frac{\text{40 mgm. yeast vitamin concentrate per kilo per day}}{\text{600 mgm. wheat embryo vitamin product per kilo per day}} = \frac{1}{15}$$

Consideration of the error of the method in our opinion justifies the following expression: the value of the ratio of potencies of these two products

may be from $\frac{1}{3}$ to $\frac{1}{7}$ with the former value to be favored as best supported by the experimental evidence. The variation alone in vitamin B requirement among different animals seems great enough to allow for such a range of values. In other words, the yeast vitamin concentrate is approximately thirteen times as potent as the wheat embryo preparation.

Some of the possible factors responsible for the variations seem to be 1, the degree of absorption of the vitamin from the alimentary canal; 2,

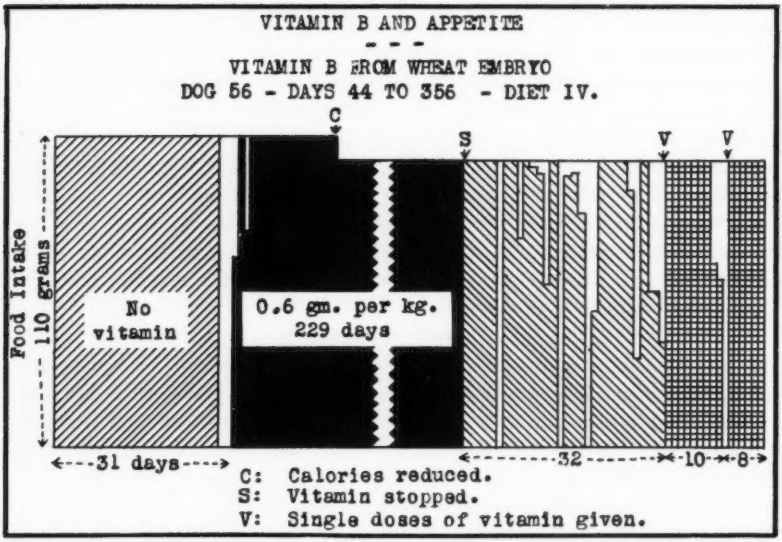

Fig. 5. The validity of the vitamin theory is well illustrated in the case of dog 56. Failure of appetite resulted after 31 days subsistence on the vitamin B-free diet. Feeding 0.6 gram per kilo of the wheat embryo preparation daily apart from the standard ration gave nutritive success lasting as long as such feeding was continued, namely, 229 days. Cessation of vitamin feeding, *S*, while keeping all other dietary factors unchanged resulted in loss of urge to eat a week later. After an irregular food intake lasting nearly four weeks, the appetite was restored promptly following vitamin administration *V*. Another failure of appetite was likewise corrected by giving vitamine *V*.

the store of this essential in the tissues at the time the animal is placed in the experiment, and the capacity of the individual to store the food factor; 3, the quantity of vitamin lost through excretory channels; and 4, a possible difference in metabolism among the animals.

It appears obvious although it has not been shown experimentally that any condition promoting cartharsis may affect the amount of vitamin B that must be provided in the food by causing a greater loss of the dietary essential in the feces. If, for example, the amount of roughage present in

such a food mixture as is shown in table 2 be increased or a change of roughage material be made, it is possible to shorten considerably the emptying time of the intestine.[13] It is quite possible, although we have not as yet investigated this point, that a greater part of the vitamin B administered under such conditions would escape absorption, and the animal would *appear* to require more of the essential than its companion given a smaller amount of roughage. In adopting such an attitude we are, of course, assuming that the vitamin contained in the feces represents that unabsorbed from the food or contained in the intestinal secretions and not that present in the cell bodies of bacteria or a result of their synthetic activity. This latter view, so far as it has been investigated, appears to be untenable (Damon, 1921; Slanetz, 1923).

It is conceivable that the ability of the intestinal wall to absorb all of the vitamin present in the food mixture may depend in part on the relative proportions of the various foodstuffs present and mutual effects exerted on one another during the process of absorption. An illustration of this is seen in the effect of the presence of the antirachitic factor on the absorption of calcium. Any differences in vitamin B requirement attributable to such causes may be slight but nevertheless large enough to account in part for the variation in the requirement which many animals show.

Data contributing to a solution of the problem of vitamin B storage in the organism have been obtained from two lines of observations: 1, examination of different organs for their content of this dietary essential (Osborne and Mendel, 1917; McCollum and Davis, 1915; McCollum and Simmonds, 1918, and Cooper, 1920); and 2, study of the behavior of animals on different diets containing varying amounts of this food factor (Osborne and Mendel,1922; Steenbock, et al, 1923; Drummond, 1918). It seems quite clear that the rat cannot store amounts of vitamin B sufficient to satisfy its need for a long period, and we believe our data justify a similar conclusion respecting the dog. In addition to what has already been written regarding this point it might be mentioned that the nutritive condition of the dog at the beginning of the experiment seems to play a rôle in determining the length of time perfect appetite is observed on the vitamin B-free ration. A fat, well-nourished animal, when placed on such a diet usually goes from three to four weeks before showing any loss of desire to eat or other sign of vitamin lack. On the other hand, dogs in an obviously inferior nutritive condition almost invariably reach the deficiency condition in a much shorter time.

It is not unlikely that the storage problem is intimately related to that of vitamin loss through excretory channels. The question as to the presence of vitamin B in the urine and feces has already been discussed.

[13] Unpublished observations of Mr. G. A. Williams in our laboratory.

Concerning the quantitative aspects of the matter it might be pointed out that Steenbock, Sell and Jones (1923) estimated that access to feces enabled their rats to get along with about one-half the amount of vitamin B that had to be supplied in the food of their experimental companions who were prevented from consuming excreta. It appears not at all unlikely that such a thing as a "vitamin B balance" exists similar in a general way to the nitrogen balance, and deserves consideration in various vitamin studies.

Suggestions that the vitamin B requirement of an organism has some relation to metabolism have already been made, notably by Dutcher (1920), Bickel (1924), Osborne and Mendel (1922), Cowgill and Deuel (1923), Cowgill, Smith and Beard (1925), and many others that might be mentioned. Additional data, to be reported in another paper, seem to throw some light on this question and to support this contention. If this be true, any marked difference in metabolism due to unusual activity or to other influences might be a factor in causing the variation in vitamin B requirement shown by the individuals in any given group of experimental animals. Further discussion of this topic we shall leave for another paper.

SUMMARY—CONCLUSION

A yeast vitamin concentrate and a wheat embryo preparation containing vitamin B were tested for their appetite-promoting power on dogs fed an artificial food mixture adequate for maintenance except with respect to vitamin B. Trials with single large doses gave results indicating that a relationship exists between the amount of the vitamin-containing material, the size of the animal in terms of the body-weight, and the number of days over which the appetite is completely restored.

The possibility that the amount of vitamin B required, when given in daily doses, may be lower than that indicated by these preliminary experiments where single large doses were given, was tested by a series of long continued feeding experiments in which the maintenance of the urge to eat was used as the criterion for the satisfaction of the animal's vitamin need. Evidence was obtained that maintenance of the desire to eat over a period of two months is not complete proof that the dog's vitamin B requirement is being entirely satisfied. Successful experiments involving periods of from three, to fourteen and fifteen months were performed, *some of these being the longest successful feeding experiments with dogs on record, so far as we are aware.* That the dietary conditions were satisfactory for maintenance in these animals was further shown by normal autopsy and detailed histological findings.

Control experiments with commercial beef extract, which does not contain vitamin B, but does promote the flow of gastric juice, showed that this substance does *not* restore the desire to eat in animals suffering from vitamin B deficiency.

From these experiments it may be concluded also that vitamin B does not owe its restorative effect on the appetite, as might be supposed, to any increased flow of gastric juice which it may produce.

The minimum amounts of yeast vitamin concentrate (Harris) and wheat embryo vitamin (Vitavose) required to satisfy the vitamin B need of dogs fed under the conditions described in this paper are approximately 40 and 600 mgm. per kilo per day respectively. These facts render it possible to feed dogs successfully on artificial diets, in which the required amount of vitamin B-containing material may be reduced to a minimum. Other sources of vitamin B may be used in this way if their potencies relative to either of these tested materials are known.

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A COMPARISON OF THE GASEOUS CONTENT OF BLOOD FROM VEINS OF THE FOREARM AND THE DORSAL SURFACE OF THE HAND AS INDICATIVE OF BLOOD FLOW AND METABOLIC DIFFERENCES IN THESE PARTS[1]

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A comparison of the gaseous content of venous blood drawn from peripheral skin vessels draining closely adjacent parts has, as far as is known to the authors, not been made. In the course of the investigations to be reported in the following papers, data were obtained which allow of a study of the variations and differences in the oxygen saturation and carbon dioxide content of blood from veins on the flexor surface of the forearm, at the bend of the elbow, and on the back of the hand or wrist.

The magnitudes of the oxygen saturation and the carbon dioxide content of the venous blood from different peripheral parts of the body should serve as an index of the respective metabolic activities of the tissues, in relation to the velocity of the blood flow through them. Such data should, moreover, give us some information as to the nature of the tissues drained by these vessels and the function of their blood supply, whether chiefly for nutrition or for temperature regulation. It may, then, be possible to interpret variations in the venous blood gas composition in two given areas, in terms of circulatory or metabolic differences. The value of this correlation is obvious.

Experimental methods. Inasmuch as the figures presented here were, in most cases, controls of experiments to be reported in other papers, to meet the needs of these experiments the arm was allowed to hang in a vertical position for slightly varying intervals of time before the blood was drawn. Also, the insertion of the needle was facilitated by the dilatation of the veins at the back of the hand when the arm was so placed. The prominence of the hand veins varies in different individuals. In a few cases it became necessary to puncture more prominent veins in the region of the radial styloid on the dorsal surface of the wrist. In drawing the blood

[1] A summary of the data of this paper was presented at the Eleventh International Physiological Congress, Edinburgh, 1923. (Quart. Journ. Exper. Physiol. Suppl. Volume, December 1923, 130.)

from the veins at the back of the hand or wrist, the needle was inserted with the point toward the fingers.

Unless otherwise stated in the protocols, the subject reclined upon a couch for at least half an hour before the blood was drawn. The arm hung down over the side of the couch during a portion of this time.

All of the subjects were apparently in good health. The blood was drawn in the morning, about one to two hours after breakfast. No attempt was made to standardize the food intake of this meal.

The blood from the antecubital fossa was taken from one of several veins in this region, the median cephalic, median basilic, the median (mediana cubiti), or the superficial ulnar, depending upon their relative prominence and ease of puncture. In some individuals the veins on the forearm lie very superficially and may be traced to the hand; in other subjects only a short branch dips out at the bend of the elbow.

In no case was a tourniquet applied in order to dilate the vessels. To preclude the possibility of obtaining static blood due to blocking of blood flow on insertion of the needle, in most of the experiments the first blood coming through the needle was discarded. This was accomplished by the use of a U-tube attached by rubber tubing to the needle and leading to two centrifuge tubes, so that the blood could be shunted into either by an adjustment of bull-dog clamps. The apparatus was essentially similar, with the exception just noted, to that already described by Van Slyke and Cullen (1). The blood was drawn under paraffin oil into tubes containing dry potassium oxalate sufficient to make a 0.3 per cent solution.

The analyses for oxygen and carbon dioxide were performed by the method of Van Slyke and Stadie (2), using the fine bore constant pressure apparatus with a mechanical shaker. We deviated from the method as described, only in that we adopted the procedure of repeatedly shaking the blood-ferricyanide mixture until the extraction of gases was complete. It was our experience that a single shaking of three minutes often failed to release all of the oxygen. This was especially true at low room temperatures. All analyses were performed in duplicate by independent observers. Two cubic centimeters of blood were used for the determination of oxygen content and capacity respectively, and 1 cc. for carbon dioxide.

Experimental. In table 1 are presented fifty oxygen determinations of blood taken from veins in the antecubital fossa of thirty-five subjects, and forty-three analyses of carbon dioxide content on thirty different individuals.

Similar data are collected in table 2. These figures represent fifty determinations of the oxygen of blood drawn from veins on the dorsal surface of the hand or wrist of twenty-five subjects, and thirty-five analyses on eighteen subjects for carbon dioxide.

The averages, at the end of each table, show that the blood from the

antecubital space (table 1) had an oxygen saturation of 70.0 per cent, an unsaturation of 6.18 volumes per cent, and a carbon dioxide content of 52.9 volumes per cent. The blood drawn from veins on the dorsal surface of the hand (table 2) had an average oxygen saturation of 87.7 per cent with an unsaturation of 2.44 volumes per cent, and a carbon dioxide content of 49.8 volumes per cent. These figures, taken from a large group of individuals under diverse conditions, show that the oxygen unsaturation of the hemoglobin and the carbon dioxide content of the blood from the veins on the dorsal surface of the hand are lower than in that drawn from the antecubital veins.

Although the average oxygen capacity of the bloods from the back of the hand and wrist was a volume per cent lower than that from the elbow, no significance can be attached to this difference. In any single individual from whom blood was drawn both from the hand and upper forearm, no difference was found in the average oxygen capacities beyond the limits of error of the methods of analysis. This conclusion is borne out by the figures for oxygen capacity in table 3.

In table 3 are collected comparisons of analyses made upon samples of blood drawn consecutively from the same individual, from veins in the antecubital space, and from the back of the hand or wrist. Here also the oxygen unsaturation and carbon dioxide content of the hand blood are lower than in that from the elbow region. The last subject (no. 8) is conspicuous in having had the lowest oxygen saturation of blood from the wrist which we encountered. Also, in this experiment the bloods from veins at the wrist and the upper forearm exhibited the least difference in their content of gases. The subject had very thick hands which were cold and clammy, and of a bluish color.

In table 4 have been compiled the number and percentage of the determinations of tables 1 and 2 which fall within arbitrary ranges of oxygen saturation. While 23 (46 per cent) of the samples drawn from the dorsal surface of the hand are between 90 and 100 per cent saturated, but 3 (6 per cent) of those from the antecubital space fall within this range of saturation. Twenty-three (46 per cent) of the bloods from veins on the forearm have an oxygen saturation below 70 per cent and only 1 (2 per cent) from the back of the hand or wrist.

The question may be raised whether the figures given above are representative of the average blood from the part. It may be argued that the veins of the hand drain only superficial skin areas, while the veins in the region of the elbow contain a mixture of blood from the skin and muscles. In table 5 are two analyses of blood, the first taken from a deep vein at the wrist in the region of the radial artery, and the second from a similar vein at the elbow close to the brachial artery. It is presumed that the bloods came from one of the venae comites of the radial and brachial arteries

TABLE 1

Oxygen content, capacity, and saturation of hemoglobin, and carbon dioxide content of blood from veins on the forearm in the antecubital fossa

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	CO_2 CONTENT	TEMPERATURE ROOM	LENGTH TIME ARM HUNG DOWN	REMARKS
			vol. per cent	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*°C.*	*minutes*	
1	Reut.	July 12, 1922	19.30	20.75	93.0	1.45	48.0	29.8	ca. 10	Right arm, very thin
2	Yud.	July 25, 1922	19.52	21.01	92.9	1.49	43.5	25.1	ca. 10	Left arm
3	Hoff.	May 10, 1923	19.27	21.35	90.3	2.08	49.8	26.1	15	Left arm. Superficial vein
4	S. G.	May 29, 1923	18.10	20.70	87.4	2.60	49.4	27.0	16	Left arm. Hand dusky
5	Baz.	November 6, 1922	16.60	19.10	86.9	2.50	53.8	26.0	15	Left arm. Superficial vein
6	Cull.	May 8, 1923	19.14	22.20	86.2	3.06		24.7	20	Left arm. Adipose individual
7	McCl.	June 5, 1923	17.81	20.74	85.9	2.93	46.4	31.0	13	Left arm
8	Latt.	June 7, 1923	17.84	21.09	84.6	3.25	58.5	32.0	20	Right arm
9	Luc.	July 28, 1922	17.55	20.96	83.8	3.41	48.2	29.0	ca. 10	Left arm. Deep vein
10	S. G.	January 27, 1923	17.24	20.69	83.3	3.45	51.7	23.0	16	Right arm. Subject seated. Same vein as nos. 11 and 27
11	S. G.	November 15, 1922	17.14	20.69	82.8	3.55	52.7	26.3	16	Right arm
12	Richt.	November 8, 1922	18.74	22.78	82.3	4.04	52.7	28.5	16	Left arm. Deep vein
13	Schee.	June 14, 1923	16.51	20.46	80.7	3.95	46.2	23.0	20	Right arm, large muscular
14	Smith	May 22, 1923	17.12	21.33	80.3	4.21	56.0	23.9	20	Right arm, very muscular
15	Fair.	May 31, 1923	15.87	20.28	78.3	4.41	48.0	23.0	22	Left arm. Veins engorged. Hand dusky. Seated
16	S. G	June 10, 1923	16.42	21.09	77.9	4.67	50.8	25.0	20	Left arm
17	Bud.	July 24, 1922	13.62	17.59	77.4	3.97	52.5	29.5	ca. 10	Left arm. Deep vein
18	A. B. L.	May 30, 1923	16.98	22.17	76.6	5.19	55.1	24.5	20	Right arm. Subject seated
19	S. G	July 21, 1922	15.01	19.72	76.2	4.71	53.3	28.7	ca. 10	Right arm

20	Bowl.	June 12, 1923	15.69	20.75	75.6	5.06	49.6	25.0	20	Left arm. Hand, forearm dusky. Albino
21	A. B. L.	June 19, 1923	16.67	22.07	75.5	5.40	54.4	26.0	20	Right arm. Hand dusky. Veins engorged
22	Lew.	June 14, 1922	16.60	22.04	75.3	5.44		24.4	2-3	Superficial vein
23	Ertr.	June 8, 1923	14.75	19.77	74.6	5.02	51.9	30.5	20	Left arm. Hand very bluish, but warm
24	Laff.	December 4, 1922	13.51	18.66	72.4	5.15		24.5	17	
25	Ad.	July 11, 1922	13.30	18.61	71.5	5.31	56.3	26.7	ca. 10	Right arm. Deep vein
26	Sem.	May 18, 1923	14.63	20.73	70.5	6.10	50.9	26.2	20	Left arm. Deep vein
27	S. G.	November 1, 1922	14.67	20.81	70.5	6.14	52.7	23.0	20	Right arm. Branch superficial vein
28	Vol.	June 1, 1923	14.97	21.64	69.2	6.67	49.3	24.0	25	Left arm. Hand dusky, forearm slightly blue
29	Adam.	May 15, 1923	13.67	19.77	69.2	6.10	55.2	23.0	22	Left arm. Deep vein
30	Laff.	November 2, 1922	12.59	18.45	68.2	5.86	53.2	23.3	10	Right arm. Deep vein
31	A. B. L.	May 1, 1923	13.77	20.44	67.4	6.67	54.2	27.0	20	Right arm. Same vein as no. 33. Seated
32	Krol.	July 29, 1922	13.06	19.56	66.8	6.50	54.6	28.0	ca. 10	Right arm. Hands cold and clammy. Deep vein
33	A. B. L.	January 24, 1923	13.79	21.06	65.5	7.27	56.1	19.5	20	Right arm. Subject seated. Vein deep
34	Trac.	June 13, 1922	13.92	21.42	65.0	7.50		27.4	2-3	Right arm
35	Gil.	July 28, 1922	12.57	19.97	63.0	7.40		28.0	ca. 10	Hands cold and clammy
36	Schee.	November 9, 1922	12.44	19.78	62.9	7.34	51.8	25.0	16	Very muscular arm, deep vein
37	And.	May 9, 1923	12.55	19.95	62.9	7.40	57.7	22.7	23	Left arm deeper vein than 44
38	McK.	July 17, 1922	12.06	19.81	60.9	7.75	55.6	28.3	ca. 10	Left arm. Deep vein
39	Beau.	June 15, 1923	12.66	21.42	59.1	8.77	53.8	24.0	20	Right arm. Fore arm, hand dusky
40	S. G.	January 31, 1923	11.78	20.54	57.4	8.76	57.0	20.5	15	Left arm. Subject seated
41	Cox	May 4, 1923	13.48	23.64	57.0	10.16	55.0	24.2	23	Left arm. Hand dusky
42	Murph.	May 2, 1923	11.10	19.52	56.8	8.42	56.8	28.1	20	Right arm. Hand bluish and cold. Seated

TABLE 1—*Concluded*

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	CO_2 CONTENT	TEMPERATURE ROOM	LENGTH TIME ARM HUNG DOWN	REMARKS
			vol. per cent	*vol per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*°C.*	*minutes*	
43	S. G.	February 5, 1923	11.64	21.13	55.1	9.49	56.2	20.0	18	Left arm. Subject seated. Hand and forearm dusky, superficial vein
44	And.	May 9, 1923	10.75	19 88	54.1	9.13	57.1	22.7	30	Left arm. More superficial vein than no. 37
45	Cates	June 13, 1922	10.71	20.67	51.8	9.96		25.0	2–3	
46	Hend.	May 23, 1923	11.07	22.14	50.0	11.07	56.8	24.6	20	Right arm. Hand very dusky. Deep vein
47	Cull.	June 13, 1923	9.39	21.47	43.7	12.08	50.2	22.0	20	Right arm. Subject seated. Veins engorged. Forearm and hand dusky
48	Lentz	May 24, 1923	8.83	20.48	43.1	11.65	55.1	23.8	18	Right arm. More superficial vein than no. 49
49	Lentz	May 24, 1923	7.93	20.49	38.7	12.56	55.1	23.8	23	Right arm
50	Cad.	June 12, 1922	7.43	19.27	38.6	11.84		31.1	2–3	Deep vein. No superficial veins
Averages			14.43	20.61	70.0	6.18	52.9	25.6		

respectively. The results are in accord with the previous findings that the venous blood from the hand has a higher oxygen saturation than that draining the forearm. Additions to the number of these experiments were not attempted because of the difficulty of obtaining the blood and the inconvenience to the subject.

It is unwarranted to assume, a priori, that blood from veins which run a very superficial course under the skin of the forearm drain a lesser proportion of the deep muscular tissue than the vessels which merely dip to the surface for a short distance at the bend of the elbow. The possibilities of anastomosis are great. If this were the case, one might expect to find that the blood from the superficial veins would have a higher oxygen saturation than that from the deeper vessels. The chances of obtaining two such bloods from one individual are slight. Usually, in any one subject, either the superficial veins preponderate and anastomose with what may be chosen as a deeper vein, or there is an absence of vessels lying close beneath the skin. We may, however, compare different subjects, of whom there are several in table 1 who showed the extreme presence or absence of superficial skin veins. Numbers 9, 12, 17, 25, 26, 29, 30, 31, 32, 33, 36, 38, 46 and 50 (table 1) belong to the group having deep veins. The average oxygen saturation of these bloods is 66.8 per cent (6.66 volumes per cent unsaturation). This is scarcely lower than the average saturation of the total fifty determinations in table 1 (70.0 per cent). Numbers 3, 5, 10, 11, 22, 27 and 43 (table 1) were taken from superficial veins, and have an average oxygen saturation of 77.7 per cent (4.66 volumes per cent unsaturation). The latter figure is significantly higher than the average saturation in table 1. It would appear from these results that blood from the more superficially situated veins may have the higher oxygen saturation. Individual exceptions to this deduction are found in the groups given above.

When one compares the oxygen saturation of blood from closely adjacent veins in the same area, differences are encountered. Numbers 48 and 49 (table 1) represent two samples drawn consecutively from different veins of the forearm. The blood from the superficial vein (no. 48) had an oxygen saturation of 43.1 per cent, and that from the deeper vein (no. 49) was 38.7 per cent. The difference in oxygen unsaturation in the two samples was 0.91 volume per cent. The carbon dioxide contents were the same in both cases. In a similar experiment the blood from the more superficial vein, no. 44 (table 1), had an oxygen saturation of 54.1 per cent, while that from the deeper vein (no. 37) was 62.9 per cent. The oxygen unsaturation of the blood from the superficial vessel was 1.73 volumes per cent greater than that from the deeper. The carbon dioxide contents were about the same. All of these bloods had a lower oxygen saturation than the average of the analyses in table 1. The experiments show the difficulty of selecting merely from their position vessels which might be

TABLE 2

Oxygen content, capacity, and saturation of hemoglobin, and carbon dioxide content of blood from veins on the dorsal surface of the hand or wrist

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	CO_2 CONTENT	TEMPERATURE ROOM	LENGTH TIME ARM HUNG DOWN	REMARKS
		1922	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*°C.*	*minutes*	
1	McCl.	August 11	19.97	20.60	96.9	0.63	47.5	24.2	5–8	Left wrist
2	Hitch.	July 18	17.02	17.69	96.2	0.67	50.0	31.8	5–10	Left wrist
3	Hitch.	July 22	16.03	16.66	96.2	0.63	48.5	28.0	5–10	Left wrist } Same vein, needle inserted at same site
4	Hitch.	July 17	17.26	18.05	95.6	0.79	49.6	27.0	5–10	Left wrist
5	Yud.	July 25	20.12	21.04	95.6	0.92	43.8	25.1	5–10	Left hand
6	Grow	August 18	18.51	19.39	95.5	0.88	47.1	31.0	18	Left hand
7	Gou.	August 16	18.42	19 37	95.1	0.95	47.0	28.9	10	Right hand
8	Grah.	July 15	16.66	17.58	94.8	0.92	51 7	24 0	5–10	Hand
9	Luc.	July 28	19 46	20 59	94 5	1.13	46.3	29.0	5–10	Left wrist. Cold clammy hands
10	Grah.	July 15	16.66	17.71	94.1	1.05	52.0	24.0	5–10	Hand
11	Mrs. S.	September 6	15.60	16.75	93.2	1.15		31.0	5	Hand
12	Ferg.	August 22	18.24	19.58	93.1	1.34	50.4	27.0	8	Left hand
13	Ferg.	August 22	18.36	19.80	92.7	1.44	50.1	27.0		Left hand, level with heart
14	McCl.	September 7	19.10	20.68	92.4	1.58	45.7	25.3	21	Right wrist. Subject seated
15	Fuch.	August 10	17.36	19.00	91.4	1.64		25.9	10	Left hand
16	McK.	July 17	18.46	20.26	91.2	1.80	47.1	28.3	5–10	Left hand
17	A. B. L.	December 21	19.49	21.38	91.2	1.89	48.9	23.0	18	Right hand
18	Dew.	June 29	19.26	21.15	91.1	1.89		26.0	5–10	Right hand
19	S. G.	July 21	18.48	20.33	90.9	1.85	50.4	28.7	5–10	Right wrist
20	Seng.	August 18	18.12	19.95	90.8	1.83		31.8	15	Left hand

		1923								
21	Hitch.	January 10	16.67	18.39	90.7	1.72	49.2	26.8	14	Right hand
		1922								
22	Seng.	August 18	18.61	20.59	90.4	1.98		31.8		Left hand. Blood drawn immediately on lowering hand
23	Bud.	July 24	16.15	17.95	90.0	1.80	51.7	29.5	5–10	Left hand
24	Rob.	September 6	15.22	16.94	89.9	1.72	47.0	27.9	10	Right hand. Subject seated
25	Swee.	December 12	18.76	20.92	89.7	2.16	50.7	23.5	34	Wrist
26	A. B. L.	December 18	18.16	20.38	89.2	2.22	47.4	23.0	8	Right hand
27	Grow	August 18	16.97	19.03	89.2	2.06	50.3	31.0		Left hand, level with heart
28	S. G.	August 12	18.43	20.76	88.8	2.33	50.6	23.0	5–10	Wrist
29	And.	August 17	17.78	20.06	88.7	2.28	53.7	29.9	15	Right hand
		1923								
30	S. G.	January 8	18.21	20.55	88.6	2.34		22.0	25	Right hand, dusky blue
31	Hitch.	January 10	16.30	18.39	88.6	2.09		26.8	14	Right hand
		1922								
32	Graf.	June 30	17.54	19.91	88.1	2.37		29.2	5	Right hand, very large and muscular
		1923								
33	McAd.	January 4	17.18	20.00	85.6	2.82	51.9		5	Right hand
		1922								
34	Rob.	September 6	14.30	16.82	85.0	2.52	48.8	27.9	10	Right hand
35	A. B. L.	December 21	18.19	21.40	85.0	3.21	47.1	23.0	34	Right wrist
36	Bud.	August 15	14.23	17.03	83.6	2.86	52.6	25.2	10	Right hand
37	Gil.	July 28	16.74	20.13	83.1	3.39		28.0	5–10	Wrist. Hands cold, clammy
38	Ad.	August 17	15.16	18.33	82.8	3.17		31.4	15	Right hand
39	Ad.	July 11	16.00	19.40	82.5	3.40	51.4	26.7	5–10	Right hand
40	S. G.	July 10	17.81	21.65	82.3	3.84	48.4	28.0	5–10	Right wrist
		1923								
41	S. G.	January 8	17.23	20.99	82.1	3.76		21.0	20	Right hand, dusky blue, veins engorged
42	S. G.	January 8	17.16	20.99	81.8	3.83		21.0	9	Left hand, less blue than no. 41

TABLE 2—*Concluded*

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	CO_2 CONTENT	TEMPERATURE ROOM	LENGTH TIME ARM HUNG DOWN	REMARKS
		1922	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*°C.*	*minutes*	
43	Mill.	June 29	16.39	20.65	79.4	4.26		27.0	10	Wrist
44	And.	August 17	16.00	20.48	78.1	4.48	55.2	29.9		Right hand. Blood drawn immediately upon lowering hand
45	Krol.	August 14	15.20	19.48	78.0	4.28		24.8	10	Right wrist. Large muscular hand
46	S. G.	December 5	15.95	20.51	77.8	4.56	53.2	24.0	30	Right hand
47	McK.	August 14	15.46	20.06	77.1	4.60	54.2	24.9	5–10	Right hand
48	Ad.	December 19	14.44	19.03	75.8	4.59		20.9	7	Right hand
49	Swee.	August 9	15.35	21.53	71.3	6.18	54.6	25.0	5–10	Wrist
50	Krol.	July 29	14.22	20.50	69.4	6.28	50.5	28.0	5–10	Right wrist. Hands cold and clammy
Averages			17.17	19.61	87.7	2.44	49.8	26.7		

TABLE 3

Analyses of the gases in consecutive samples of blood from veins in the antecubital space and on the dorsal surface of the hand or wrist

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	CO_2 CONTENT	TEMPERATURE ROOM	SITE BLOOD DRAWN
		1922	vol. per cent	vol. per cent	per cent	vol. per cent	vol. per cent	°C.	
1	Yud.	July 25	20.12	21.04	95.6	0.92	43.8	25.1	Left hand
			19.52	21.01	92.9	1.49	43.5		Left forearm
2	Luc.	July 28	19.46	20.59	94.5	1.13	46.3	29.0	Left wrist
			17.55	20.96	83.8	3.41	48.2		Left forearm
3	McK.	July 17	18.46	20.26	91.2	1.80	47.1	28.3	Left hand
			12.06	19.81	60.9	7.75	55.6		Left forearm
4	S. G.	July 21	18.48	20.33	90.9	1.85	50.4	28.7	Right wrist
			15.01	19.72	76.2	4.71	53.3		Right forearm
5	Bud.	July 24	16.15	17.95	90.0	1.80	51.7	29.5	Left hand
			13.62	17.59	77.4	3.97	52.5		Left forearm
6	Gil.	July 28	16.74	20.13	83.1	3.39		28.0	Wrist
			12.57	19.97	63.0	7.40			Forearm, same side
7	Ad.	July 11	16.00	19.40	82.5	3.40	51.4	26.7	Right hand
			13.30	18.61	71.5	5.31	56.3		Right forearm
8	Krol.	July 29	14.22	20.50	69.4	6.28	50.5	28.0	Right wrist
			13.06	19.56	66.8	6.50	54.6		Right forearm

TABLE 4

Summary of the number and percentage of the determinations in tables 1 and 2 which fall within different ranges of oxygen saturation

SITE BLOOD DRAWN	O_2 SATURATION OF HEMOGLOBIN PER CENT				
	100–90	90–80	80–70	Below 70	
Dorsal surface of hand or wrist	23	19	7	1	Number falling in each group
	46.0	38.0	14.0	2.0	Per cent of total in each group
Antecubital space forearm	3	11	13	23	Number in each group
	6.0	22.0	26.0	46.0	Per cent of total

TABLE 5

Oxygen content, capacity, and saturation of hemoglobin of blood from deep veins of the wrist and forearm

SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	SOURCE BLOOD
	1924	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	
McK.	June 11	17.36	20.35	85.3	2.99	One of venae comites, radial artery
Ad.	June 12	15.12	18.97	79.3	3.85	One of venae comites, brachial artery

TABLE 6

Average oxygen saturation and room temperature of upper and lower halves of tables 1 and 2

ANTECUBITAL SPACE		DORSAL SURFACE OF HAND OR WRIST	
O_2 saturation	Room temperature	O_2 saturation	Room temperature
per cent	*°C.*	*per cent*	*°C.*
81.3 (3.85 vol. per cent unsat.) (Nos. 1–25 inclusive)	26.5	92.9 (1.37 vol. per cent unsat.) (Nos. 1–25 inclusive)	27.5
58.7 (8.50 vol. per cent unsat.) (Nos. 26–50 inclusive)	24.6	82.3 (3.53 vol. per cent unsat.) (Nos. 26–50 inclusive omitting no. 33)	25.9

Average oxygen saturation at room temperatures below and above (including) 26°C.

TEMPERATURE ROOM	ANTECUBITAL SPACE		DORSAL SURFACE OF HAND OR WRIST	
	O_2 saturation	Average temperature	O_2 saturation	Average temperature
	per cent	*°C.*	*per cent*	*°C.*
Below 26°C.	66.4 (7.02 vol. per cent unsat.) (28 dets.)	23.5	86.3 (2.77 vol. per cent unsat.) (20 dets.)	23.6
26°C. and above	74.6 (5.11 vol. per cent unsat.) (22 dets.)	28.2	88.9 (2.20 vol. per cent unsat.) (29 dets.)	28.8

suspected of draining principally skin or deep areas. They illustrate the point that blood from neighboring veins, under the same external conditions, may show significant differences in oxygen saturation.

Inasmuch as the environmental temperature is known to effect the venous oxygen saturation (3), (4), it becomes of interest to examine our figures from this point of view. In each case the room temperature is noted in the protocols (tables 1 and 2). The subject was always allowed to rest in the room for a period of about 30 minutes before the sample was taken. Hence, it may be assumed that vascular adjustments to the room temperature had taken place.

The data are arranged in two ways. First, on the assumption that the differences in the oxygen saturation in the upper and lower halves of the two sets of figures (tables 1 and 2) are due solely to temperature, a comparison may be made of the average room temperature of each group of analyses with the oxygen saturations. Such a summary is given in the first part of table 6, where it is noted that the average room temperature in the experiments recorded in the first halves of tables 1 and 2 is higher than in the second portions. For room temperatures differing by 2°C., the antecubital blood showed an oxygen saturation of 81.3 per cent in the first 25 analyses and 58.7 per cent in the second, a deviation of 4.65 volumes per cent in the oxygen unsaturation. The blood from the back of the hand in the first half of table 2, with a higher average room temperature of 1.6°C., has an oxygen saturation of 92.9 per cent as compared to 82.3 per cent for the remaining determinations. This is a difference of 2.16 volumes per cent in the oxygen unsaturation of the two portions of the analyses. This compilation, taken at face value, indicates that the variations of oxygen saturation observed in tables 1 and 2 are, partially at least, associated with the temperature of the environment. The effect seems to be less marked upon the hand bloods.

A second method of analyzing the effect of temperature is to separate the figures into groups according to the room temperature. In the latter part of table 6 is given the average oxygen saturation of these bloods divided again into two portions, the first taken at room temperatures below 26°C., and the second at temperatures of 26°C. and above. This is approximately the average of the temperature range in these experiments, and roughly divides the total number of determinations. Here again the higher oxygen saturations are obtained at the higher room temperatures, but the effect upon the blood at the back of the hand is almost nil. The blood from the antecubital veins had an oxygen saturation of 66.4 per cent at room temperatures below 26°C., and a saturation of 74.6 per cent for 26°C. and above. Thus, at the higher room temperatures the oxygen unsaturation is lower by 1.91 volumes per cent. By this method of compilation the samples from the dorsal surface of the hand or wrist have oxy-

gen saturations of 86.3 and 88.9 per cent, a variation in oxygen unsaturation of but 0.57 volume per cent in the two temperature ranges. This last figure is barely beyond the limits of the error of the methods of analysis.

It will be observed (table 6) that the differences in oxygen saturation at room temperatures above and below 26°C. are about one-third those obtained by averaging the figures in the upper and lower halves of tables 1 and 2. Since the average room temperature difference is more than twice as great in the former grouping (5°C.) than in the latter (2°C.), it is obvious that the deviation in oxygen saturation in the first and second portions of tables 1 and 2 is not entirely due to the environmental temperature.

Additional evidence substantiates the conclusion just made. In the same individual it is found that venous blood, drawn on different occasions from the identical site of puncture, at equal room temperatures may have very different oxygen staturations. Or, the room temperature may vary considerably, and the oxygen saturations may be the same. This is illustrated in table 1. Numbers 10 and 27 have respective oxygen saturations of 83.3 and 70.5 per cent, yet the room temperature, time of day the blood was drawn, and the previous food and activity were alike. In this same individual (S. G.), samples 10 and 11 were taken from the identical vein and site of puncture, the oxygen saturations of the bloods were the same, but the room temperature differed by 3°C., on the two days. A similar result is shown on subject A. B. L. Numbers 31 and 33 (table 1) drawn from the same vein and point of puncture, at room temperatures differing by 7°C., show but little difference in oxygen saturation. This topic will be discussed later in the paper.

Another point seems worthy of statement here. Respiratory quotients of 0.3 to 3.5 are derived from the results in table 3, where bloods from the antecubital region and the back of the hand are compared in consecutively drawn samples. Aside from experiment 1 (table 3), which showed no distinguishable amount of oxygen lost or carbon dioxide taken up in the passage of the blood through the forearm, it is evident that the contents of these two gases in the venous blood from a peripheral part of the body may vary independently.

Discussion. The evidence presented above shows that the average oxygen saturation of the venous blood from the hand is higher and the carbon dioxide content lower than in blood from veins of the forearm. Lundsgaard and Möller (5) have found that the oxygen content of "cutaneous blood" obtained from an incision in the skin of the finger is almost identical with arterial blood. Although a large number of the bloods from veins at the back of the hand have an oxygen saturation within the range of arterial blood, the average of the fifty determinations (table 2) is below this level.

It becomes of interest to speculate as to the cause of the relatively high

oxygen saturation and low carbon dioxide content of the venous blood from the hand. A low utilization of oxygen by the tissues of this part is one of the first factors to suggest itself. That such may be the case seems reasonable when one considers the structure of the hand. It is, for the most part, a bony skeleton covered with skin, with but a slight amount of tissue that is highly metabolic. The muscles responsible for the movements of its parts are situated principally above the wrist. The hand is predominantly made up of skin tissue and its metabolism is, therefore, relatively lower than that of the muscular forearm.

Since the hand has such a large skin area in proportion to its mass (6), and one of the chief functions of the skin is to regulate body temperature, its blood circulation becomes of prime importance. Hewlett and Van Zwaluwenburg (7) have shown that the blood flow in the hand, relative to its volume, is faster than the flow in the combined forearm and hand. Inasmuch as the speed of the blood flow is a factor in determining the extent of the gaseous exchange per unit amount of blood, one would expect that the hand blood would differ from that from the upper forearm in gaseous content. A rapid blood flow and a low metabolism, or a rapid flow in proportion to the degree of metabolism, would be a still more favorable condition for a slight gaseous exchange between each unit amount of blood and the tissues. Lundsgaard and Möller (5) suggest a similar explanation to account for the high oxygen content of "cutaneous blood."

One purpose of the rapid flow of blood through the hand may be for the regulation of body temperature, in accord with the general function of the skin circulation. The total flow is faster, on this basis, merely because the greater part of the circulation is in the skin of this member. Quite another function may be ascribed to the phenomenon. If, as stated above, the hand is an area of low oxygen consumption due to the paucity of tissue capable of a high level of metabolism, then possibly it is inherently incapable of maintaining its own temperature by its intrinsic heat formation. Therefore, it must depend in great measure upon the heat of the blood passing through it, which in turn would necessitate a comparatively rapid flow of blood.

For such a mechanism to be adequate, there should be a continuous flow through the smaller vessels of this part, of a nature so as to offset minor external influences which would lead to a cooling of the hand. This would require an arrangement of vessels somewhat different from that ordinarily found in the skin. Hoyer (8) has suggested that a vascular anomaly which would serve precisely such a function is to be found in the direct connections between arteries and veins, the so-called arterio-venous anastomoses, which are alleged to be very numerous in the finger pads of man. It may also be pointed out as pertinent to this discussion, that the blood flow through the capillaries at the base of the nail bed of the fingers is practi-

cally continuous, in contradistinction to other skin areas observed (9). Stewart (6) makes the interesting observation that, in proportion to their bulk, the blood flow in the fingers is more active than in the hand as a whole.

Compared to other peripheral parts of the body studied, the tissues of the hand apparently are peculiar in respect to the relation of blood flow to rate of metabolism. Stewart (6) finds that the blood flow through the hand per unit mass may be twice the amount which passes through he foot under the same conditions. A comparison of the gaseous content of blood from the foot with that from the hand (10) is in accord with this statement.

It was found, although not invariably, that blood from veins which run a superficial course under the skin throughout most of their length in the forearm and hand may have a higher than average venous oxygen saturation, or a higher saturation than blood from deeper veins in other individuals. This may be taken to indicate that the blood in these vessels is largely drawn from skin areas, relatively low in metabolism, or, at least, low as compared to the blood flow. The superficial position and the readiness with which these veins respond to external temperature causes one to wonder whether one of their functions may possibly be to participate in the heat elimination and conservation in the body. A dilatation of these vessels would bring a large volume of blood close to the surface of the skin; a constriction would have the contrary effect.

It has been pointed out that two bloods from the same individual, drawn consecutively from the arm and hand, may show more or less independent variations in their oxygen and carbon dioxide contents. The cause of this is not known, but it may be due to a chance collection of blood at a time when the tissues were in a phase of the metabolic process where oxygen was being consumed and little carbon dioxide given off, or vice versa. Whatever its cause, this fact, along with the observation that even closely adjacent vessels may have blood of different gaseous content, shows the futility of attempts to obtain reliable respiratory quotients from a computation based upon gases of blood from arteries and peripheral veins.

An analysis of the data contained in the protocols has indicated that external temperature is a factor in causing the variability of the gases in venous blood. The differences in temperature ordinarily encountered in a room (20° to 30°C.) have but little effect upon the oxygen saturation of the blood drawn from veins on the back of the hand, while they may cause considerable deviation in that from the forearm. The oxygen which passes into the tissues from each unit volume of blood upon exposure to cold is greater in the forearm than in the hand. The question arises whether this finding indicates a difference in the response to temperature of the blood flow through these regions. The data in this paper show that

the metabolism in the hand is low, at least in proportion to the rate of blood flow in the vessels. For this reason the speed of the circulation may possibly be somewhat reduced without causing the blood to suffer any considerable reduction in oxygen content. Since the volume of the oxygen loss is so slight it would appear that there may be another explanation of this phenomenon. Perhaps the hand, due to its more continuous exposure to the environment, has adapted itself so that its blood flow is not affected over the narrow range of variations of room temperature. This may be another way of stating what has already been said above, that the necessity for a continuous flow through the hand has been met by an anatomical adjustment of the smaller vessels, such that the flow of blood through them is not so markedly influenced by slight changes in external temperature.

From the differences brought out by the two arrangements of figures in table 6, and from an inspection of table 1, where high and low room temperatures are noted at both extremes of the table, we are led to believe that factors other than temperature are involved as a cause of the variations in oxygen saturation. Although precautions were taken to eliminate the effects of previous activity or exposure to temperatures different from that prevailing in the room, it is impossible to be certain that these influences did not persist at the time the blood was drawn. Nevertheless, we venture to suggest that at least part of the variation in the gaseous content of peripheral venous blood is closely related to the cause of cold clammy hands and skin in some individuals, as contrasted to the warm dry skin in others. It has been our experience—a few cases are designated in the protocols of table 2—that the former group usually has an oxygen saturation of venous blood below the average. A diminished rate of blood flow has also been observed in the arms and hands of individuals with cold hands, as compared to the flow in those whose skin is habitually warm (11), (6). There suggests itself, as a causative agent, something of the nature of a central vasomotor phenomenon.

As stated previously, all of the bloods in tables 1 and 2 were drawn after the arm had hung in a vertical position for a period of time. Under these conditions, the arm and hand veins became more or less distended, and a dusky bluish hue was evident on the back of the hand and wrist. Since this condition might be considered as an indication of a blood stasis in the arm, it becomes necessary to determine whether the average venous blood gas content has been affected. Some evidence on this point may be obtained by comparing our figures with those procured under different conditions by other observers. Figures for the gaseous content of antecubital blood are available. Lundsgaard (12) performed 18 determinations of venous oxygen on 11 different individuals. The blood was drawn from the cubital vein, "while the arm rested comfortably at the side of the body on a small moderately soft pillow." His figures show an average

oxygen content of 13.6 volumes per cent, an unsaturation of 6.0 volumes per cent, and a saturation of 69.4 per cent. Our results in table 1 give an average oxygen content of 14.5 volumes per cent, an unsaturation of 6.1 volumes per cent, and a saturation of 70.0 per cent. Lundsgaard also found wide variations in the same subject.

Harrop (13) reports 15 analyses of normal venous oxygen and carbon dioxide of blood from the bend of the elbow. The average oxygen content of these bloods is 14.04 volumes per cent, the unsaturation 6.14 volumes per cent, the saturation 69.5 per cent, and the carbon dioxide content (average of 10 determinations) 54.7 volumes per cent. These figures also agree closely with those in table 1.

Hence, in so far as this evidence may be indicative, hanging the arm, with resultant engorgement of the veins, has not affected the average oxygen saturation or carbon dioxide content of the blood from the veins of the forearm. Further experiments and more detailed discussion of this point will be included in a subsequent paper (10).

SUMMARY

1. The oxygen saturation of the hemoglobin of the blood from veins on the dorsal surface of the hand or wrist is higher, and the carbon dioxide content lower, than that of blood taken from veins of the forearm in the antecubital fossa. This is a reflection of a difference in the degree of the metabolism of the tissues, in relation to the rate of blood flow through the respective parts drained by these vessels. Hypotheses which account for the necessity for a rapid circulation of blood through the hand, and speculations upon the mechanism of accomplishment are presented.

2. The more superficial veins of the forearm may have a blood of a higher oxygen saturation and a lower carbon dioxide content than deeper veins. This points to the former blood as being chiefly from the skin.

3. Closely adjacent veins of the forearm may exhibit differences in their content of blood gases.

4. The carbon dioxide and oxygen contents of two bloods, drawn consecutively from the hand and the bend of the elbow, may vary independently. The failure to obtain satisfactory respiratory quotients from arterial and peripheral venous blood may be thus accounted for.

5. One cause of the variability of the gas content of peripheral venous blood is the effect of external temperature upon the blood flow through the part. The content of gases in the blood from veins on the back of the hand appears to be less influenced by this factor than is the blood from the forearm. Probable reasons are given.

6. Variations in the gaseous content of venous blood are also produced by causes arising within the body. The suggestion is made that the responsible agent is associated with the influences which cause cold clammy

hands in some individuals and warm dry skin in others, possibly a central vasomotor effect.

7. Hanging the arm vertically, with the development of venous engorgement, seems to have little effect upon the average gaseous content of the blood from veins of the forearm.

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THE EFFECT OF LOCAL TEMPERATURE UPON THE PERIPHERAL CIRCULATION AND METABOLISM OF TISSUES AS REVEALED BY THE GASEOUS CONTENT OF VENOUS BLOOD[1]

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Recent experiments (1), (2) have shown that, when an arm is subjected to heat, the oxygen saturation of the venous blood increases. Exposure to cold, on the contrary, diminishes the oxygen saturation of the blood in the veins (1), (2).

These results agree perfectly with the known effect of heat and cold upon the color of the skin and on the blood flow. The human skin, subjected to high temperatures of air or water, takes on a pink color; the volume of the part exposed and the rate of blood flow through it are increased (3), (4), (5), (6), (7), (8). Cold produces a pale or blue color of the skin; the volume of a limb and the velocity of blood flow through its vessels are diminished (3), (4), (5), (6), (7), (8).

The tacit conclusion which investigators have drawn from these observations is that the vaso-dilatation and the oxygen saturation of the blood increase progressively with the degree of heat, and likewise diminish with cold (1), (2).

Evidence is presented in this paper which shows that such a general deduction is unwarranted, especially in regard to the action of cold. The existence is established of certain factors which lead to the appearance of a pink color of the skin, an increased oxygen saturation, and a decreased carbon dioxide content of the venous blood upon exposure of a part to low temperatures. Varying temperatures of cold water produce marked qualitative differences in the composition of the venous blood gases.

That the human skin may take on a bluish hue when exposed to cold air is a matter of common experience. The data to be presented here show that, in an isolated area such as the arm, in the absence of shivering, the blueness is not caused by the lowest temperatures, but is dependent for its existence upon intermediate temperatures consistent with the continuance of metabolism in the tissues.

[1] The results of the experiments in this paper were presented at the Eleventh International Physiological Congress, Edinburgh, 1923. (Quart. Journ. Exper. Physiol., Suppl. Volume, December, 1923, 130.)

The erroneous generalizations made are attributable partly to the indiscriminate use of water and air as the surrounding temperature mediums in studying the effects of cold upon the skin circulation, color, or venous blood gas content. A comparison of these two mediums separately studied shows that they may produce quite varying effects. The gaseous content of the venous blood and the color of the skin may differ radically when the arm is surrounded by water or air of the same temperature. It becomes apparent that an important variable involved in these studies is the actual temperature of the tissues of the part, which may be quite dissimilar at a given temperature of air and of water.

An attempt will be made to fix the limiting ranges of temperature producing the different changes in the blood gases and in skin color. With a knowledge of these factors, certain conclusions can be derived as to the influence of temperature upon the state of the blood vessels and the metabolism in a given isolated area.

Experimental methods. The data are chiefly concerned with analyses of the gaseous content of blood drawn from veins on the flexor surface of the forearm, in the region of the bend of the elbow (the antecubital fossa). In a few experiments the blood was taken from veins on the dorsal surface of the hand.

Unless otherwise stated in the protocols, the subject was comfortably seated in a chair. The arm, hung down, was immersed up to the elbow into water at the desired temperature. The controls and experimental bloods were obtained under the same conditions in respect to posture and approximate length of time during which the arm was allowed to hang. They were drawn from the same vessel, and when possible from the identical site of puncture.

In most cases, no attempt was made to maintain the water at a constant temperature, hence it varied by 1 to 3°C. during the course of an experiment. The highest and lowest points reached are given in the protocols. The water was stirred more or less continuously.

The blood, in all cases, was drawn without stasis or exposure to air. The arm was removed from the water only a distance sufficient to allow insertion of the needle into the vein. The technique of drawing the blood and the methods of analysis have been described in the preceding paper (9).

Whenever more than one experiment was performed upon a subject at a single sitting, the fact is indicated by cross references in the protocols. An ample interval of time between such experiments was allowed for the complete return of the local circulation to its state at room temperature. If the arm was exposed to very high or very low temperatures, the effects of which may persist for a long time, the experiment was either the last of a series, or was performed alone.

TABLE 1

Effect of immersion of the forearm and hand into water of different temperatures (6 to 46°C.) upon the oxygen content, capacity, and saturation of hemoglobin, and carbon dioxide content of blood from veins in the antecubital space (forearm)

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	CO_2 CONTENT	TEMPERATURE WATER	TIME ARM EXPOSED	TEMPERATURE ROOM	CHANGE IN SATURATION FROM NORMAL	REMARKS (COLOR SKIN)
			Arm in water at 6 to 18°C.									
		1923	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	°C.	*minutes*	°C.	*per cent*	
		January 31	11.78	20.54	57.4	8.76	57.0		15	20.5		All bloods from same vein
1	S. G.	February 2	19.40	20.79	93.4	1.39	50.8	5–7	18	23.0	+37	Pink hyperemia
		February 5	11.64	21.13	55.1	9.49	56.2		18	20.0		Skin dusky
		February 6	11.95	20.58	58.0	8.63	57.1		17	4–4.5		Skin arm pale, hand bluish
		January 23	11.82	23.14	51.1	11.32	57.7		15	8.5–7		All from same vein. Skin dusky
2	A. B. L.	January 24	13.79	21.06	65.5	7.27	56.1		20	19.6		
		January 25	19.47	21.71	89.7	2.24	49.8	5.5–8.0	20	22.9	+24	Pink hyperemia
3	Murph.	May 2	11.10	19.52	56.8	8.42	56.8		20	28.1		Skin dusky
			16.90	18.83	89.7	1.93	53.6	8.5–5.5	16	26.0	+33	Pink hyperemia
4	Adam.	May 15	13.67	19.77	69.2	6.10	55.2		22	23.0		
			17.23	19.64	87.7	2.41	53.4	7.5	19	23.0	+19	Pink hyperemia
			16.51	20.46	80.7	3.95	46.2		20	23.0		Large muscular arm
5	Schee.	June 14	15.26	20.85	73.2	5.59	48.9	7.5–9.0	20	23.7	−8	Pink hyperemia
			14.67	21.23	69.1	6.56	48.1			23.9		Dusky hyperemia 12 minutes after removal from water

6	Bowl.	June 12	15.69	20.75	75.6	5.06	49.6		20	25.0		Albino. Skin dusky
			17.40	20.68	84.2	3.28	49.7	8.5–9.5	21	25.6	+9	Pink hyperemia
7	Cull.	June 13	9.39	21.47	43.7	12.08	50.2		20	22.0		Skin dusky
			14.89	20.57	72.4	5.68	48.5	8.5–10.5	20	23.0	+29	Pink hyperemia
			8.53	20.92	40.8	12.39	50.0			23.5		Fore arm pale blue 27 minutes after removal from water
		1922										
8	Baz.	November 6	16.60	19.10	86.9	2.50	53.8		15	26.0		Reclined
			16.61	19.10	87.0	2.49	52.8	10.5–9.5	21	26.0	0	Pink hyperemia
9	Laff.	November 2	12.59	18.45	68.2	5.86	53.2		10	23.3		Reclined
			12.91	18.45	70.0	5.54	56.5	15.5–11	30	23.3	+2	Pink hyperemia
10	S. G.	November 1	14.67	20.81	70.5	6.14	52.7		20	23.0		Reclined
			15.41	20.76	74.2	5.35	52.4	15.5–12.5	22	23.0	+4	Pink hyperemia
		1923										
11	S. G.	November 15	17.14	20.69	82.8	3.55	52.7		16	26.3		Reclined
			17.19	20.52	83.8	3.33	51.8	14.5–16.5	20	26.3	+1	Slightly pink
12	Murph.	May 2	11.10	19.52	56.8	8.42	56.8		20	28.1		Skin dusky
		June 21	17.34	19.94	87.0	2.60	50.4	14–17.5	22	30.0	+30	Slightly pink. See nos. 21, 23.
13	A. B. L.	January 24	13.79	21.06	65.5	7.27	56.1		20	19.5		
		January 29	14.68	20.63	71.2	5.95	54.5	15.5–17	17	21.0	+6	Slightly pink. See no. 22
		1922										
14	Schee.	November 9	12.44	19.78	62.9	7.34	51.8		16	25.0		Reclined. Large muscular arm
			10.53	19.92	52.9	9.39	52.5	16.5–17	21	25.0	−10	Skin pale, not blue

TABLE 1—*Continued*

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	CO_2 CONTENT	TEMPERATURE WATER	TIME ARM EXPOSED	TEMPERATURE ROOM	CHANGE IN SATURATION FROM NORMAL	REMARKS (COLOR SKIN)
			vol. per cent	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*°C.*	*minutes*	*°C.*	*per cent*	
Arm in water at 6 to 18°C.—Continued												
		1922										
15	Richt.	November 8	18.74	22.78	82.3	4.04	52.7		16	28.5		Reclined. Large muscular arm
			16.49	22.53	73.2	6.04	54.0	16–18	16	28.5	−9	Slightly dusky
		1923										
16	Bowl.	June 12	15.69	20.75	75.6	5.06	49.6		20	25.0		Albino, slender arm. Skin dusky
			16.23	20.80	78.0	4.57	47.4	17.5–18.5	24	25.6	+2	Slightly pink. See no. 6
Arm in water at 18 to 39°C.												
17	S. G.	May 29	18.10	20.70	87.4	2.60	49.4		16	27.0		Skin dusky
			16.23	20.76	78.2	4.53	55.5	18–19	21	28.5	−9	Mottled pink, See no. 27
		1922										
18	Laff.	December 4	13.51	18.66	72.4	5.15			17	24.5		Reclined
			10.43	18.66	55.9	8.23		18.5–19	30	24.5	−17	
		1923										
19	Latt.	June 7	17.84	21.09	84.6	3.25	58.5		20	32.0		
			13.63	21.01	64.8	7.38	60.7	17.5–19.5	23	31.9	−20	Normal pale color. See no. 26
20	Fair.	May 31	15.87	20.28	78.3	4.41	48.0		22	23.0		Large muscular arm. Skin dusky
			9.89	20.13	49.2	10.24	53.1	18.5–20	21	24.0	−29	Pale pink
21	Murph.	June 21	12.77	19.16	66.6	6.39	53.6	19.5–21.5	20	30.0		Slightly dusky. See nos. 12, 23

22	A. B. L.	January 29	13.69	19.90	68.8	6.21	53.5	22.5–23	16	21.0		Skin pale. See no. 13
23	Murph.	June 21	15.15	19.34	78.4	4.19	51.3	26.5–28.5	20	29.5		Normal color. See nos. 12, 21
24	A. B. L.	May 30	16.98	22.17	76.6	5.19	55.1		20	24.5		
			10.82	21.86	49.6	11.04	53.9	28.5	28	25.5	−27	Skin pale
25	Cox	May 4	13.48	23.64	57.0	10.16	55.0		23	24.2		Skin dusky
			10.53	23.30	45.2	12.77	52.7	29.5–28	24	25.0	−12	Normal color
26	Latt.	June 7	17.84	21.09	84.6	3.25	58.5		20	32.0		
			14.86	20.68	71.9	5.82	56.2	28.5–29	24	31.3	−13	Normal pale color. See no. 19
27	S. G.	May 29	18.10	20.70	87.4	2.60	49.4		16	27.0		Skin dusky
			18.83	20.51	91.8	1.68	49.7	28.5–29.5	21	28.7	+4	Normal pale color. See no. 17
28	S. G.	June 10	16.42	21.09	77.9	4.67	50.8		20	25.0		
			11.73	20.52	57.2	8.79	53.4	29	20	24.0	−21	Skin pale. See no. 36
29	Beau.	June 15	12.66	21.42	59.1	8.77	53.8		20	24.0		Skin dusky
			9.76	20.56	47.5	10.80	56.0	29	29	24.6	−12	Skin pale, cold. See nos. 35, 42
30	McCl.	June 5	17.81	20.74	85.9	2.93	46.4		13	31.0		
			18.57	20.76	89.5	2.19	46.8	29.5	22	31.5	+4	Skin pale. Water felt cool
31	Fair.	May 31	15.87	20.28	78.3	4.41	48.0		22	23.0		Skin dusky
			11.03	20.30	54.4	9.27	49.9	30 –29.5	22	24.5	−24	Arm pale, hand dusky. See no. 20
32	Ertr.	June 8	14.75	19.77	74.6	5.02	51.9		20	30.5		Skin dusky
			15.74	19.70	79.9	3.96	50.4	29.5–30	24	31.0	+5	Normal skin color
33	A. B. L.	May 30	16.98	22.17	76.6	5.19	55.1		20	24.5		
			13.30	21.84	60.9	8.54	51.7	33.5–33	24	26.1	−16	Skin pale. See no. 24

TABLE 1—*Concluded*

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	CO_2 CONTENT	TEMPERATURE WATER	TIME ARM EXPOSED	TEMPERATURE ROOM	CHANGE IN SATURATION FROM NORMAL	REMARKS (COLOR SKIN)
			Arm in water at 18 to 39°C.—*Continued*									
		1923	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*°C.*	*minutes*	*°C.*	*per cent*	
34	A. B. L.	June 19	16.67	22.07	75.5	5.40	54.4		20	26–27		Skin dusky
			17.11	21.41	80.0	4.30	53.0	35–34.2	20	27.5–28	+5	Color normal. See no. 41
35	Beau.	June 15	12.66	21.42	59.1	8.77	53.8		20	24.0		Skin dusky
			13.48	20.64	65.3	7.16	54.8	36–35	20	25.0	+6	Temperature indifferent. Color normal. See nos. 29, 42
36	S. G.	June 10	16.42	21.09	77.9	4.67	50.8		20	25.0		
			14.36	20.61	69.6	6.25	51.1	36.5–35.3	21	25.0	−8	Color normal. See no. 28
		1922										
37	Seng.	July 31	13.57	20.43	66.4	6.86		40.5–37.5	11	28.5		
38	S. G.	July 21	15.01	19.72	76.2	4.71	53.3		10	28.7		
		August 1	14.14	20.50	69.0	6.36	53.6	40.5–37.5	11	28.5		
			Arm in water at 40 to 46°C.—*Continued*									
39	Key.	August 8	18.59	21.34	87.1	2.75	48.6	41.5–39	10	29.8		
40	Rob.	August 7	14.09	16.37	86.1	2.28	50.6	42.5–39.5	10	28.6		
		1923										
41	A. B. L.	June 19	16.67	22.07	75.5	5.40	54.4		20	26–27		Skin dusky. See no. 34
			21.01	22.03	95.4	1.02	45.8	42.7–40	21	28.5	+20	Subject sweat. Slight hyperemia
			21.35	22.16	96.4	0.81	46.1	47–45	21	29.0	+21	Red hyperemia. Sweat
42	Beau.	June 15	12.66	21.42	59.1	8.77	53.8		20	24.0		Skin dusky
			19.75	20.47	96.5	0.72	49.6	45.5–42.5	20	25.3	+37	Red hyperemia. Subject sweat

The subject was always allowed to rest for sometime with the bare arm exposed to room temperature before the first sample of blood was drawn. Since the results are comparative, factors of diet and time of day were not controlled.

In the experiments performed in a cold room, during the period of exposure, the subject clothed and with the arm bare was seated in a chair.

Experimental. The analyses in table 1 have been arranged to show the effect, upon the oxygen saturation of the hemoglobin and the carbon dioxide content of blood taken from veins of the upper forearm, produced by immersing the forearm and hand into water of increasing temperatures (6 to 46°C.). The results have been divided into three groups which will be discussed separately and in relation to each other.

Arm in water at 6 to 18°C. In the first series of 16 experiments (6 to 18°C.) it will be noted that, in comparison with the normals at room temperature, the oxygen content and saturation of the hemoglobin was increased, and the carbon dioxide content of the blood decreased. The lower the temperature, the more marked was this effect. Only three of the results showed a significant change in oxygen capacity of the hemoglobin (nos. 2, 3 and 7). These changes are neither great nor numerous enough to allow of any deductions.

The only decreases in oxygen saturation are found in experiments 5, 14 (subject Schee.) and 15 (subject Richt.). Both of these subjects were athletes, especially chosen to test whether the effect of cold upon the venous blood gases would be modified by an unusually large mass of muscular tissue on the forearm. The results indicate that variations in tissue mass of the exposed area will undoubtedly be a factor in causing at least quantitative variations in effect. Assuming a limit of depth to which the cold or heat may penetrate, with varying bulk of the arm in the several subjects different proportions of the total mass of tissue and vascular bed would be affected. One would expect this factor to be most noticeable at the border line between any two ranges of temperature in table 1, because it would be less strongly masked by the extremes of effect. As an example, subject Bowl (no. 16), an albino with a small forearm, in contrast to the two athletes (nos. 14 and 15) just mentioned, showed an increase in the oxygen saturation of his blood after immersion of the arm in water at a slightly higher average temperature.

For reasons which will be stated later, it is of interest to determine whether the temperature of the room may be a factor in modifying these results obtained when the arm was exposed to cold water. An inspection of the protocols (table 1) shows that similar effects may be obtained at a room temperature of 23°C. (nos. 4 and 10) or 30°C. (no. 12); nor are the exceptions to the rule just mentioned to be ascribed to an influence of the surrounding atmospheric temperature.

The color of the skin of the immersed portion of the arm is of interest in connection with these results. Far from exhibiting the "cyanosis or blueness" of cold, the skin acquired a vivid pink hyperemia which, at the lower temperatures, was very marked and was of uniform intensity over the exposed area.

This hyperemia persisted for some time after removal of the arm from the water. The pink coloration of the submerged part made a sharp line of demarkation with the normal skin at the point of contact of air and water. The pinkish tint became less marked at temperatures above 15°C., but was still present at 18°C. (no. 16), or slightly higher. Occasionally, at the latter temperature the skin presented a somewhat mottled appearance of pale and pink areas (no. 17), or was pale (no. 14) with a slight suggestion of blueness (no. 15).

No tendency was ever noted for the skin to become blue before the appearance of the pink color. The onset of the coloration was rapid, and appeared to pass directly from the normal skin tint. The pinkish hue persisted as long as the arm was immersed in the cold water; frequently the observations were continued for thirty minutes. In a few cases, blood was drawn from veins on the back of the hand (table 2), after a short exposure ($\frac{1}{2}$ to 3 minutes) to cold water, with no evidence of a preliminary drop in oxygen saturation, but with quite the contrary effect (nos. 3 and 5, table 2).

Upon removal from the water, the arm exposed to room temperatures and not exercised, may assume a dusky bluish appearance. In two experiments, nos. 5 and 7 (table 1), blood was drawn after removing the arm from the water, at a time when the appearance of a bluish color was noted. In both experiments the oxygen content and saturation of this blood was lower than that of the sample drawn while the arm was immersed in the water, and also lower than the preliminary control. The contrast in oxygen saturation of the two experimental bloods is especially marked in no. 7, where the last sample was taken 27 minutes after removal of the arm from the water.

Arm exposed to air below 8°C. In experiments 1 (February 6) and 2 (January 23, table 1), in addition to the control bloods taken at ordinary room temperature, samples were also drawn after the subjects had been seated in a room the temperature of which was at a low level comparable to that of the water. In both experiments, the oxygen saturation of the hemoglobin was markedly lower, and the carbon dioxide content of the blood higher, in the sample drawn from the veins of the bare arm exposed to an atmospheric temperature below 8°C., than in the blood from the arm immersed in equally cold water. Neither while seated in the cold room nor when the arm was immersed in water did the subjects shiver. As compared to the specimen drawn at the higher room tem-

perature (20°C.), the figures in experiment 1 for the colder air (below 5°C.) showed no significant change. In experiment 2 the oxygen saturation was still lower and the carbon dioxide content higher in the colder room (below 8°C.) than in the warmer (20°C.). The oxygen capacity of the blood (23.1 volumes per cent) of subject A. B. L. (expt. 2) was increased in the sample drawn in the cold room. This figure is the highest of many similar determinations made on this subject.

In these experiments in which the arm was exposed to a cold atmosphere, the color of the skin differed radically from that in cold water of about the same temperature. In the water, as stated before, the skin was pink. When exposed to cold air, the upper forearm was pale or slightly dusky blue, while the hand was more decidedly of a dusky bluish color. The veins of the forearm and hand were constricted in both cases. These points will be discussed further in this paper.

Arm in water at 18 to 39°C. The second group of figures (table 1) represent experiments in which the arm was introduced into water ranging in temperature from 18 to 39°C. and show, in general, a decrease of the oxygen saturation of hemoglobin, and a rise in the carbon dioxide content of the blood drawn from veins on the forearm. This change in the blood gases was apparent between 19 to 30°C. of the water, but most marked in the lower portion of this range. In several instances, an individual at one sitting had an arm exposed to different temperatures in this group (nos. 17, 19, 21, 28 and 29), with the result that the oxygen saturation of the blood, though in absolute value still below the control at toom temperature, tended to rise as the temperature of the water increased.

It is very noticeable among these figures, that the oxygen content of two bloods may vary independently of the carbon dioxide content. Although a fall in the oxygen content was usually accompanied by a rise in the carbon dioxide, there are many exceptions to be found in the table. This discrepancy has been observed and previously commented upon by the authors (9).

Of the 17 experiments in this group (18 to 39°C.) with normal controls, five show a slight but significant increase in oxygen content and saturation of the hemoglobin; this is a larger number of deviations from the rule than was seen in an equal number of experiments in the preceding temperature area (6 to 18°C.).

One probable cause of these variations in effect has already been mentioned, namely, differences in tissue mass. In this particular range, it would appear that the temperature of the room is also a modifying influence. A study of the protocols shows that up to 29°C. of the water, the room temperature differences (21 to 32°C.) are not related to any certain qualitative or quantitative alterations in the effect of temperature

upon the venous blood gases. At 29°C. and above, however, such a relationship is revealed. Comparing experiments 27 to 30 inclusive, where the average temperature of the water was approximately the same, it will be noted that when the room temperature was high, 28.7° and 31.5°C. (nos. 27 and 30), the oxygen saturation of the blood was slightly increased. On the other hand, at lower room temperatures, 24 and 24.6°C. (nos. 28 and 29), there was a marked diminution of the oxygen saturation of the blood. A similar correlation is seen in experiments 31 and 32, with a room temperature of 24.5 and 31°C. respectively. The first blood (no. 31) showed a marked fall in oxygen content, the latter (no. 32) a slight rise. At higher temperatures in this area, unfortunately, the room temperatures did not differ greatly, so that an analysis of the results from this point of view is not possible. The probable causes of this phenomenon will be discussed later in the paper.

Compared to the controls, four of the figures for oxygen capacity (nos. 28, 29, 34 and 35) were slightly but significantly decreased.

The color of the skin when exposed to temperatures of 18 to 39°C. varied. At 18 to 19°C. there was apparent, in some subjects, a mottling of pale and pink spots, in others, a pale pink suggestive of the higher temperatures of the preceding group. For the most part, the skin of the forearm was paler than normally. The hands were of a bluish hue, which one may designate as the "blueness of cold." The latter color was most often observed below the mid-region (20 to 25°C.) of this temperature range, where the water felt cool and the veins were constricted. At higher temperatures of the water (34 to 36°C.) the skin was of a normal pale color rather than pink. The sensation upon placing the hand in water at these temperatures was indifferent and the superficial veins were more prominent.

Above 39°C. the water became lukewarm, the skin at times pinkish, and the veins still more dilated than at the lower temperatures. There was considerable variation in the temperature sensations described above, depending upon whether the skin was warm or cold at the time of entering the water.

Arm in water at 40 to 46°C. In the last group of experiments in table 1, in which the arm was placed in water at 40 to 46°C., the oxygen saturation of the hemoglobin tended to rise and the carbon dioxide content to fall. At the higher temperatures the oxygen saturation rapidly approached that of arterial blood. The few determinations given may be taken as typical of the results to be expected.

Although the oxygen saturation of the blood rapidly approached and even rose above a normal value at about 35°C., in general, a marked increase was not found until a temperature of the water of 39 to 40°C. was reached. A sharp definition of these points is impossible as variations in different individuals are inevitable, for reasons already stated.

To eliminate, to a great extent, the influence of variations in tissue mass, several experiments have been performed using blood from the veins on the dorsal surface of the hand. Since the hand is mainly a skin area, with but little muscle tissue, and the bulk of the vascular bed is relatively close to the surface of the skin, one would expect that at any given temperature a more uniform result would be obtained in different individuals. It would be exceedingly difficult to study the whole range of temperature effect upon blood from veins of the hand, for the reason that insertion of a needle into these vessels, when constricted, offers but few chances of success.

The results in table 2 show that although the oxygen saturation of the blood might be high at water temperatures of 39 to 42°C., it was still below the level of arterial blood. However, at a temperature of 44 to 46°C. we have shown (10) that the blood taken from the veins at the back of the hand is indistinguishable in oxygen and carbon dioxide content from arterial blood. It would appear that when the hand is exposed to water in the region of 44°C., there is a marked alteration of conditions in certain vessels which allows the arterial blood to pass so rapidly into the veins, that for each unit volume the gaseous content is practically unchanged.

In water at high temperatures (44 to 46°C.) the skin assumed a purplish-red color, quite different from the pink of cold. At milder degrees of heat there was a pinkish hyperemia. The veins became extremely dilated as the temperature of the water rose.

General results. In order to represent graphically the several points which have been described above, in figure 1 have been plotted the differences between the oxygen saturation of the hemoglobin in the blood of the control sample and that drawn when the arm was exposed to different degrees of temperature (table 1). It will be noticed that temperatures of the water, up to about 18°C., result, for the most part, in an increase in the oxygen saturation of the blood. Between 18 and 39°C., on the other hand, there is a decrease in oxygen saturation. In the region above 39°C., the oxygen saturation again rises above the normal figures.

It will be seen that there is a tendency to a variation in effect, in the region of 18°C., and from 29 to 36°C., as evidenced by points above and below the zero line of oxygen saturation (the normal control).

A somewhat larger number of points representing the oxygen saturation of the hemoglobin of blood from veins of the forearm after exposure to water at different temperatures were plotted in figure 2. The averages set down in table 3 were then plotted, and a curve drawn through them. One point stands out clearly on this figure. The greater number of values for the oxygen saturation of the blood obtained after immersion of the arm in water at temperatures below 18°C. and above 40°C. lie

TABLE 2

The oxygen content, capacity, and saturation of hemoglobin of blood from veins on the dorsal surface of the hand before and after exposure to water of different temperatures

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	CO_2 CONTENT	TEMPERATURE WATER	TIME EXPOSED	TEMPERATURE ROOM	REMARKS
			Hand in water at 13 to 15°C.								
		1922	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*°C.*	*minutes*	*°C.*	
1	McK.	August 14	15.46	20.06	77.1	4.60	54.2			24.9	Control
			18.75	19.85	94.5	1.10	51.6	13.5–15	15	24.9	After hand removed from water
		1923									
2	Hitch.	January 10	16.30	18.39	88.6	2.09				26.8	Control
			16.01	17.87	89.6	1.86		12.5–15.5	10	26.0	Only fingers immersed
		1922									
3	S. G.	August 1	17.13	20.38	84.1	3.25	51.5		1½	28.5	Hand in warm water
			17.53	19.95	87.9	2.42	54.3		2–3	28.5	Hand in ice water 2 to 3 minutes
			Hand in water at 39 to 44.5°C.								
4	Graf.	June 30	17.54	19.91	88.1	2.37				29.2	Control
			18.36	19.77	92.9	1.41		40.5–37.5	24	29.0	
5	Swee.	August 9	15.35	21.53	71.3	6.18	54.6			25.0	Control. Blood from wrist
			17.96	21.18	84.8	3.22	52.6	40.5–37.5	10	25.0	
			18.18	21.35	85.1	3.17	52.1			25.0	Hand in ice water ½ to 1 minute

6	Mill.	June 29	16.39	20.65	79.4	4.26				27.0	Control. Blood from wrist
			18.46	19.79	93.4	1.33		41.5–38.5	15	27.0	
7	Bud.	August 15	14.23	17.03	83.6	2.80	52.6			25.2	Control
			14.86	17.03	87.3	2.17	51.4	41.5–38.5	15	25.2	
8	Fuch.	August 10	17.36	19.00	91.4	1.64				25.9	Control
			18.00	19.08	94.4	1.08		42.5–41	10	25.9	
9	Hitch	January 10	16.30	18.39	88.6	2.09				26.8	Control
			17.04	18.39	92.7	1.35		44–41.5	12	26.0	
		May 3	16.05	16.57	96.8	0.52	48.4	45–44	15	24.5	Donor for transfusion two days previously

above the average oxygen saturation of fifty bloods drawn from the same area at room temperature (9). This occurred regardless of the initial oxygen saturation, which might have been very low, and in spite of any other existing conditions tending to antagonize the result.

Deviations above and below the average normal oxygen saturation are also discernable in figure 2 at water temperatures between 18 and 20°C. and 28 to 39°C. Although no great exactness is claimed in this connection, the curve as drawn crosses the line of average normal oxygen at

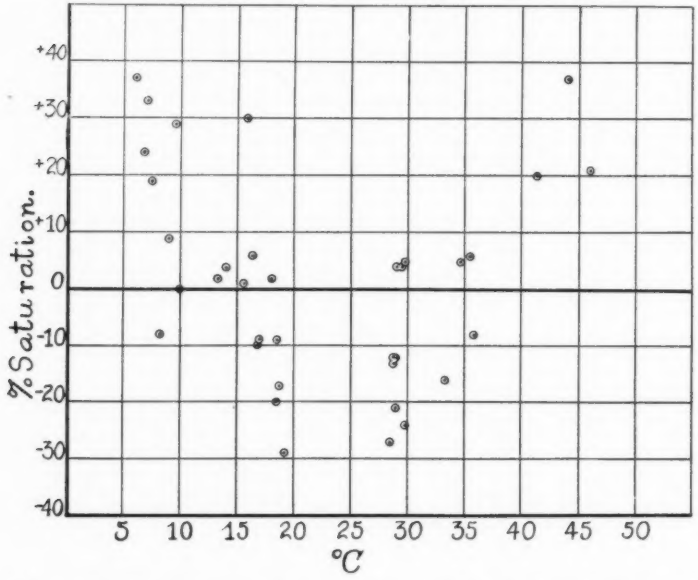

Fig. 1. Differences between the oxygen saturation of the hemoglobin in the blood of control samples and in the blood drawn after the arm was exposed to water of various temperatures.

Abscissa: temperature of the water. Ordinate: the percentage oxygen saturation difference, above (+) or below (−) the control figure.

16°C. and between 34 and 35°C. This latter point is of the order of or slightly above exposed skin temperature (11). As previously stated there is evidence to indicate that the oxygen saturation of the blood, at temperatures above that of the skin, rises more slowly than the curve in figure 2 indicates, suddenly increasing rapidly at about 44°C. of the water.

Table 3 gives a comparison of the average oxygen saturations of the blood obtained from the forearm when surrounded by various temperature ranges of water (table 1), and when exposed to air. The latter figures are in part taken from data in a preceding paper (9).

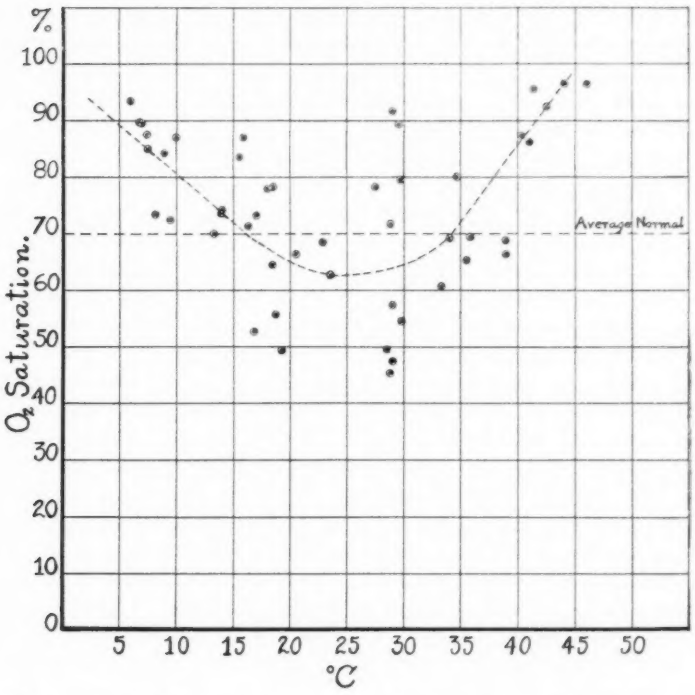

Fig. 2. Showing the oxygen saturation of blood from veins of the forearm after immersion into water of various temperatures.

Abscissa: temperature of water. Ordinate: percentage saturation of the hemoglobin. ⊙ Separate experimental points. ⊗ Averages of groups of figures from table 3.

TABLE 3

Average oxygen saturation of the hemoglobin of blood from veins on the forearm after exposure to different temperatures of water or air

ARM IN WATER			ARM IN AIR		
Number of experiments	Temperature of water	O_2 saturation Hb.	Number of experiments	Temperature of air	O_2 saturation Hb.
	°C.	*per cent*		°C.	*per cent*
8	5–10	85			
8	10–18	74			
11	18–29	63	2	4–8	55
12	29–39	69	28	20–26	66
			22	26–32	75
5	39–46	92			

The figures for the average oxygen saturation of the blood drawn at room temperatures of 4 to 8°C. correspond best to those obtained with the arm in water at 18 to 29°C., while those taken at room temperatures ranging from 20 to 26°C. approximately agree with the values resulting from placing the arm in water at about 29°C. Temperatures of the room from 26 to 32°C. give a saturation of oxygen in the venous blood of the forearm which is found when the temperature of the surrounding water is in the region of 39°C. With the figures at present available, it is not possible to place these corresponding ranges more definitely. Certain it is that as the water surrounding an arm is progressively cooled, the oxygen saturation of the venous blood first shows a decrease, then an increase. The arm exposed to cold air fails to exhibit the secondary rise in oxygen saturation at the temperatures used in these experiments.

Discussion. In searching for the probable causes of the increase in oxygen saturation of the hemoglobin, and the decrease of the carbon dioxide content of the venous blood of the arm exposed to temperatures of water below 18°C., two possibilities arise. First, the cooling of the tissues may depress their metabolism, an effect common to chemical processes, and one known to occur in poikilothermous animals (12). Second, a cooling of the blood in the part may result in a depression of the dissociation of oxy-hemoglobin (13), (14), hence decrease the oxygen tension available for the muscular oxidative processes. The final effect of both of these factors would be a diminution of the gaseous exchange in the limb, made evident by a decreased oxygen consumption and carbon dioxide elimination by the tissues.

Such a marked reduction in oxygen utilization, as is reflected by the increased oxygen content of the blood from the veins of the upper forearm, which undoubtedly obtain a part of their supply from the deeper tissues, must indicate that the cold penetrates to a considerable depth, affecting a large portion of the tissues and the vascular bed. The work on the rabbit of Macleod and his collaborators (15) showed that a local application of cold to the skin might cause a reduction in temperature for some distance beneath the surface. The maximal depth of penetration was not determined, but a marked fall of temperature was detected at 11 to 15 mm. beneath the surface of the thigh, when the cold application was 21 to 23.5°C. lower than the temperature of muscle. Lefèvre (16) found in man that when needles were inserted into the skin brought in contact with water below 18°C., the temperature at the aponeurosis was 24.7°C., rising rapidly from this point until at a distance of 8 to 10 mm. beneath the surface the temperature of the tissue was normal.

A penetration of the cold from all sides of a cylindrical surface such as the arm, up to the deep fascia covering the muscles and for a short distance beyond, with a fall in temperature of the order found by Lefèvre,

would involve a cooling of a large portion of the tissues. As a result, the stability of the oxy-hemoglobin would be increased. The degree of temperature necessary to reduce the processes associated with heat production in the muscles, in vivo, cannot be conjectured from data at present available. It must be emphasized again here that, under the conditions of our experiments, shivering never occurred.

Our observations upon the color of the skin agree closely with those of Lefèvre (17) practically throughout the temperature range in table 1. This author insisted, apparently against much opposition, that at 5°C. of water there was an instant and marked hyperemia of a carmine tint, which increased for 2 to 3 minutes, was limited to the regions immersed, and persisted for periods of an hour in the bath. At 12°C. there was still an immediate hyperemia which diminished in intensity above this point. At 18°C. it was feeble and developed slowly, fading between 20 to 25°C. From 25 to 35°C. Lefèvre designated the area of anemia, but towards 36 to 37°C. a hyperemia again began, until finally with hot water the color became a rose-red.

As to the state of the local circulation accompanying the pink hyperemia of cold, we disagree radically with the views expressed by Lefèvre. He states that the hyperemia of cold reveals the existence of a rapid and strong circulation as active as with heat. As evidence, he says that the paleness produced by pressure on the skin, "la tache pâle," disappears with equal rapidity in the hyperemia of heat or of cold. Although our evidence does not rest upon this point, it has been our experience that, while the time given by Lefèvre, namely, 2 seconds, may be of the order necessary for the refilling after cold, the time for the paleness to disappear with an active hyperemia from heat was about a half second.

That the pink hyperemia of cold is not associated with a rapid blood flow is indicated by the following evidence. When the circulation in the forearm and hand was impeded by means of an inflated blood-pressure-cuff to a point where the radial pulse was absent, the forearm and hand became rapidly cyanosed. In one illustrative experiment, the blueness was definite in several trials within 1.8 minutes after applying the pressure. If now the hand and forearm were immersed in water at 7°C. until the skin was brightly pink (4 minutes), and allowed to remain after the circulation of the arm was again cut off, the pink color of the immersed portion of the skin persisted as long as the subject could bear the pain of the combined procedures, which in this case was for 4 minutes. At this time the skin above the point of immersion, but below the cuff, was intensely cyanosed. Other experiments of this type showed always a delay in the onset of the cyanosis when the arm was pink with cold, even at higher temperatures of water than 7°C. Similar experiments performed upon an arm hyperemic from heat showed no delay in the appearance of the blue color.

Another analogous type of experiment of significance here is the following. When the circulation through the forearm was stopped, and time allowed for the development of a cyanosis, the arm introduced into water at 5 to 6°C. remained blue, with the exception of a few areas over the knuckles and the first phalanges. If, after one or two minutes of immersion, the pressure was released from the cuff, instantly the portion of the skin which was exposed to the water took on a vivid and persistent pink color, while the part which was not in the water still retained a bluish hue. The pink color developed under these conditions was far more intense than the after-flush which sometimes follows such a procedure.

Again, with a pressure of 40 mm. of mercury maintained by a cuff on the arm until a mild blueness developed, upon immersion in cold water, the exposed part became pink, separated by a sharp blue line at the point of contact of air and water.

Further indirect evidence upon this point is submitted. When the arm was immersed in hot water (44 to 46°C.), there was often a distinct general feeling of warmth to the extent that the subject perspired at distant parts. Never, in any of our experiments, with the arm in cold water, did the subject either experience a general feeling of cold, or shiver. The latter effect might have been in evidence if the blood flow had been equally rapid in the two cases. Shortly after removal of the arm from the cold water, the subject usually experienced a chill and momentarily shivered, coincident with a sensation of cold in the axilla. Apparently the shivering was caused by the passage of cool blood from the arm, as circulation began to return to normal. Also, the blood flow from the veins, through a needle, appeared much slower when the arm was in cold water than when exposed to room temperature, and certainly slower than when immersed in hot water. Furthermore, the glass vessel into which the blood from a vein of a cold arm was drawn, felt cool to the touch, quite in contrast to the normally warm blood.

Robinson and Stiles (18) affirm that they found an increased heat elimination from the hand subjected to extremely low temperatures. Hewlett (19) states that, "in most persons" he was, "unable to obtain an increased blood flow during exposures to water sufficiently cold to cause considerable pain." In our experience such a degree of cold would produce a pink hyperemia.

Direct observations of capillary flow in the human skin under the influence of cold have been made. Carrier (8) describes the following train of events:

"The hand was put in water at 10°. For four minutes there was little change in the contracted capillaries . . . . for the next six minutes the capillaries gradually relaxed and filled with arterial blood, while the

hand became red . . . . after 10 minutes the blood stream . . . . stopped. The hand became blue and all the capillaries, on the back of the hand as well, were open and filled with venous blood." The author goes on to state that, " . . . . the arterioles are so completely contracted that the flow of blood stops. The open capillaries fill with venous blood and the skin is cyanotic." With this last statement our results are at complete variance.

Bruns and König (20) on the contrary, found that with the arm and hand in water at 10°C., after 4 to 5 minutes, a hyperemia recognizable macroscopically as well as microscopically could be seen. The capillaries were open and the blood stream moved forward. This interval compares favorably with the time of development of the maximum hyperemia from cold in our experiments.

In view of the evidence presented, there can be little doubt that in the "pink hyperemia" of cold, as at higher temperatures associated with the paleness or blueness of cold (5), (6), the rate of blood flow is decreased.

This conclusion is not incompatible with the causes for the increased oxygen saturation which we have suggested above. A slow blood flow should increase the amount of oxygen extracted by the tissues from each unit volume of blood, provided that the call for oxygen is present and the oxy-hemoglobin is able to dissociate. Since the oxygen saturation of the blood is increased, it must be concluded that either or both of the latter factors are inactive; therefore, the slowing of the flow merely facilitates a further cooling of the part.

The state of the blood vessels in a part under conditions of its exposure to cold, is of interest. The veins are obviously markedly constricted and one gets the impression from their "wiry" nature, that this is an active process rather than a passive collapse due to a small inflow of blood. The very prompt response of a superficial vein to a localized application of heat or cold adds to this belief. Additional facts contribute to the conception that the arterial side of the vascular bed is constricted and the blood flow decreased. Mosso (3) and others have found a diminution in the arm volume, Stewart (5) and Hewlett (19) a diminished blood flow, and Lommel (21) an acceleration of the arterial wave under the influence of cold on the arm.

Mosso (3), from a single experiment, concluded that at a low temperature of water (below 7°C.) the arm exhibited a secondary swelling in the plethysmograph, which he attributed to a paralysis of the vessels, including veins and arteries. So far as is known to the writers, this observation has never been confirmed. More recently (8), (27), the paralysis has been confined to the skin capillaries and venules. By the use of the plethysmographic method, we were unable to show a secondary increase of arm volume when the skin was pink with cold. It is, indeed, true that after

the hand has been exposed to low temperatures of water, especially below 10°C., it feels engorged and swollen, and movement is spastic. Hewlett (19) is inclined to regard this condition as due to a "dilatation of the cutaneous capillaries and venules with edema."

In three experiments we made comparative measurements at various points on the immersed and opposite hand. In two cases a slight enlargement was detected in the hand exposed to cold. The fact that the immersed parts were pink showed that an engorgement existed in certain vessels, whose blood gives color to the skin. Simultaneous constriction of arteries and veins might have well masked this effect in plethysmographic measurements.

That the direct action of the cold may have a paralyzing action upon the capillaries and venules of the skin cannot be refuted. However, in view of the relatively high temperature at which the development of the pinkness of the skin in cold water may occur (18°C.), we are led to believe that factors other than paralysis contribute to the capillary and venule dilatation.

Assuming that the veins may be under the control of a veno-pressor mechanism for which there is some evidence (22), and that the constriction of these vessels on exposure to cold is due to such an active process, there arises the possibility that, if arterial inflow is not entirely cut off, the capillary and venule pressure will be increased. Measurements of capillary pressure under these conditions have been made. Hough and Ballantyne (23) reported an increase in pressure in the capillaries back of the nail bed of the finger, upon exposure to cold air at 6°C. or to cold water of from 5 to 9°C. In the latter case they state that the characteristic red glow was present. The veins were almost invisible. Danzer and Hooker (24) were not able to subscribe to these results. Nevertheless, under circumstances such as described, the possibility of an increased resistance on the venous side of the vascular bed exists. As a consequence, there may occur a venule and a capillary dilatation, for experimental evidence indicates that the capillary pressure is related more directly to that in the veins than to that in the arteries (7), (24), (25), (26). If the tone of the capillaries were diminished by the cold, the dilating effect of the increased venous resistance upon them would be intensified. The capillaries and venules would thus be engorged with blood of a low oxygen unsaturation. Hence, the pink color of the skin due to extreme cold would be accounted for.

In regard to the effects occurring at a temperature range of water between 18 and 39°C. there is a greater unanimity of opinion. In passing from the higher temperature (39°C.) to the lower (18°C.), the blood flow through the part is reduced (6). The arterioles, capillaries, venules (8), (27) and veins become constricted and the volume of the arm diminished

(3), (4). We have shown that the venous blood from an arm exposed to this range of water temperature has a maximum oxygen unsaturation and carbon dioxide content. The skin may be pale (8), (27) or blue. Obviously, the state of the vessels whose blood gives color to the skin is not the same in the two conditions. In the case of the pale skin the vessels responsible for the color are constricted, while with the blue skin they are relatively dilated.

In view of the fact that the superficial veins are visibly contracted when the arm is immersed in water at the lower temperatures of this range (18 to 25°C.), there also exists here the possibility that the increased resistance in them may lead to a dilatation of the venules and capillaries. Since the blood contains a large amount of reduced hemoglobin, the color of the skin is blue. When pale, it may be assumed that the pressure of the venous constriction is not sufficient to produce a venule and capillary dilatation.

In this connection the work of Briscoe (28) is of interest. In a study of patients whose hands were blue or readily became so in cold weather, she found no alteration in venous pressure, but a capillary pressure above that of normal controls. In other experiments, in response to a reflex constriction produced by placing one hand in cold water, the venous pressure as well as the capillary pressure in the opposite hand of such patients showed a tendency to rise. As the veins were scarcely visible in many of these subjects, the author admits that changes in venous pressure were difficult to follow. Briscoe concluded that the increase in capillary pressure was due to a venule constriction. The analogy of this conclusion to our hypothesis regarding the cause of the blueness of cold in normal individuals is obvious. We have placed the resistance in the markedly contracted veins, a condition which we believe is the result of a true venous pressor effect of cold.

It has already been pointed out that when the arm is exposed to room temperatures of 5 to 8°C., or surrounded by water of from 18 to 29°C., the color of the skin may be the same in the two cases and the gaseous content of the blood may agree closely. This phenomenon finds its explanation in the relative temperatures of the tissues. The evidence to follow indicates that they are probably the same. Lefèvre (16) has shown that the skin surrounded by water tends to assume its temperature for some distance beneath the surface. In cold air, even in the absence of shivering, Benedict and his co-workers (11) have observed that the temperature on the surface of the skin remains above that of the air for long periods of time. Different parts of the body show varying degrees of susceptibility in this respect. The experiments in table 3 demonstrate that when the arm is exposed to very cold water, the gaseous exchange between the blood and the tissues is diminished. Comparable tempera-

tures of air failed to cool the arm to this degree. The possibility, however, remains that such an effect might be produced if the atmospheric temperature were sufficiently reduced. It may be that the pink cheeks, ears and noses often seen in very cold weather are counterparts of the reaction of the tissues in very cold water. It is, therefore, apparent that the protective mechanisms in the subcutaneous tissue, are more effective against cold air than against water of equal temperature. In the former case, the tissue temperature is still high enough for the continuance of oxygen consumption. Heat continues to be produced at relatively short distances beneath the skin, with the result that the temperature of the whole part tends to remain above that of the surrounding air. The body in cold air throws a warm dry blanket about itself. Hewlett (29) states that a person with a half naked body felt indifferent to room temperature of 22 to 24°C., and warm to 27 to 31°C. Obviously, when a large enough surface of the body is exposed to cold, the added protective factor of shivering still further increases the heat production and warms the surface. The factors of the degree of motion and humidity of the air undoubtedly play a rôle in determining the extent to which the skin is cooled. Our results have all been obtained in rooms where the movement of the air was comparatively slight.

Just as cold water is most effective in cooling the body, it is also highly probable that hot water is superior to air in warming the tissues and dilating the blood vessels.

Under the influence of heat, 40 to 46°C., it is agreed that the volume of the part increases and the blood flow is accelerated (3), (4), (5), (6), (7), (8). The red hyperemia is associated with dilatation of all the blood vessels (7), (8), (30). So marked are these effects that we have found that the oxygen saturation of the venous blood taken from the back of the hand, may be indistinguishable from that of arterial blood. The maximum increase in the oxygen content and saturation of the venous blood would appear to start in the neighborhood of 42 to 44°C. It is not possible to state at this time whether this represents a critical temperature at which dilatation of certain vessels (arteries) occurs, or one at which a maximum dilatation of vessels (arterioles, capillaries and venules) that are already partially opened at lower temperatures takes place. A figure illustrating the action of temperature upon local blood flow published by Hewlett et al (6) shows a sudden rise in rate at about 40°C. and a maximum flow at a temperature somewhat above 40°C. Mosso (3) on the basis of plethysmographic observations states that hot water may produce a vascular paralysis. Hewlett and his collaborators add further evidence in support of this idea. It is of interest to note that Lewis (30) reports the presence of a capillary pulse when the hand is immersed in water at 45 to 47°C.

Throughout all of the temperature ranges discussed in this paper, it may be stated that, in general, the degree of temperature seems to be a more pertinent factor in the production of a given result, than does the time of exposure.

It has been noted above that occasional variations occur in the response of the oxygen saturation of the blood to a given temperature. Two factors concerned have been mentioned, namely, differences in the mass of tissue in the part exposed, and in room temperature. The influence of the latter was most apparent at 29 to 34°C. or slightly higher; this may be considered to be within the ordinary range of variation in skin temperature on exposed parts (11).

It has been known for a long time that, when a superficial part of the body reacts to local vasomotor temperature effects, there is usually a transmission of a like response to other areas. This is indicated by changes in volume (31) or in blood flow (32), (6). We have confirmed these observations by plethysmographic methods, and, in addition, have found that when the water in the plethysmograph was at the extremes of temperature, i.e., below 20°C. or above 40°C., reflex temperature effects transmitted to the arm in the plethysmograph, by placing the opposite member in hot or cold water, were slight or absent. In our experiments, unmistakable and most definite reflex responses were obtained when the plethysmograph arm was surrounded by water of a temperature of from 25° to 34°C.

This phenomenon might have played a rôle in causing variations in the effect of temperature upon the oxygen saturation of venous blood, when the arm was immersed in water at about 29°C., at relatively high and low room temperatures. Undoubtedly, similar results might have been obtained at other temperatures of water between 25 and 34°C., at different room temperatures.

It is reasonable to assume that 29°C. is below the average temperature of the skin of the arm at the room temperatures existing in our experiments (24 to 31°C.). The forehead may have an external temperature of 31.6°C., when exposed to an atmospheric temperature of 17°C. (11). Immersion, then, in water at 29°C. might be expected to give a vasoconstriction in the arm with decreased rate of blood flow and diminished oxygen content. However, if the rest of the body were warm and compensation by heat elimination were necessary, the reflex mechanism from distant areas might be able to "break through" the local effects of water at about 25 to 34°C. As a result the vessels might remain dilated at least to their former degree. On the other hand, in a relatively cooler atmosphere, with the body tending to conserve heat by peripheral vasoconstriction, the effect of a local cooling would be additive.

These results suggest an antagonism between pressor and depressor

influences resulting in an algebraic summation of reflex effects (33). A somewhat similar phenomenon is quoted by Hewlett (6). He states that it has been found that the effect of psychic influences noted in plethysmographic studies were most apt to be encountered when the blood flow approximated the normal rate, and tended to diminish when the water surrounding the arm was either hot or cold. Hewlett and his collaborators (6) found that heat applied to an arm increased the rate of blood flow through it, and that similar changes might occur in the opposite arm, not exposed to the heat. When the subject was kept cool or chilled the rate of blood flow in the arm not directly affected might not be accelerated.

SUMMARY

1. When the forearm and hand are immersed in cold water below 18°C., the skin takes on a pink hyperemia, the oxygen saturation of the hemoglobin increases, and the carbon dioxide content decreases in the blood drawn from veins in the antecubital fossa.

a. This phenomenon is caused by a depression of the gaseous interchange between the blood and the tissues of the arm, either by a suppression of the oxidative processes of the tissues, or by a reduction in the dissociation of oxy-hemoglobin, or both.

b. Evidence is presented which indicates that the blood flow through the vessels under these conditions is diminished.

c. Arterioles and veins are actively contracted, while the vessels whose blood gives color to the skin are dilated. It is suggested that the latter phenomenon finds its explanation in an increased resistance in the veins which causes in the venules and capillaries a heightened pressure and a passive dilatation. The latter is facilitated by a decrease in capillary tone due to cold.

2. At higher temperatures of water, 18 to 39°C., the venous blood reaches a minimum of oxygen saturation and a maximum of carbon dioxide content, indicating that metabolism in the tissues is active.

a. It is within this range of water temperature that the first suggestion of the paleness and blueness of cold is noted.

b. The veins and arterioles are relatively constricted; blood flow is diminished. The vessels whose blood gives color to the surface are either constricted or dilated, thus imparting respectively a pale and a bluish hue to the skin. An increased pressure from a high venous resistance may also be the cause of the dilatation of capillaries and venules in this case. However, it is the increased oxygen unsaturation of the capillary blood which is responsible for the blue color.

c. The development of a blue color of the skin from exposure to cold can take place only at temperatures which produce vaso-constriction

and decreased blood flow through the part, but which allow the continuance of oxidative processes in the tissues and an adequate dissociation of oxy-hemoglobin.

3. The oxygen saturation of the hemoglobin and the carbon dioxide content of the blood rapidly approaches the level of these gases in arterial blood, when the arm and hand are surrounded by water at a temperature of 40 to 46°C.

a. The skin is of a reddish or purplish color at the higher temperatures in this range.

b. Evidence is discussed which points to a dilatation of all parts of the vascular bed.

c. There exists a critical temperature, 42 to 44°C., at which the maximum increase in the gaseous content of venous blood begins.

4. The effect upon the blood gases of room air at a temperature of 5 to 8°C. compares closely in its effects to water of 18 to 29°C.

The explanation would appear to be that the skin and subcutaneous tissue tends to assume the temperature of the surrounding water; the temperature of the tissues surrounded by air is maintained at a high level. The normal factors of resistance against heat loss are effective.

5. Two causes which account for variations from the usual response in the gaseous composition of the venous blood of the arm surrounded by water at different temperatures are:

a. Differences in the mass of arm tissue.

b. Atmospheric temperature. In substantiation of the latter factor, plethysmographic experiments are cited which show that the atmospheric temperature, in affecting the general state of the peripheral skin vessels, may "break through" or take precedence over a certain restricted range of local temperature effect of water. This was most apparent in our experiments when the water temperature was about 29 to 34°C. or slightly higher. The mechanism simulates algebraic summation of reflex effects.

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A CYANOSIS, UNRELATED TO OXYGEN UNSATURATION, PRODUCED BY INCREASED PERIPHERAL VENOUS PRESSURE[1]

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When an arm is allowed to hang vertically from the shoulder and kept stationary, there develops a greater or lesser degree of engorgement of the veins of the forearm and hand. The skin of the hand, wrist and lower part of the forearm takes on a bluish color of varying intensity.

Since cyanosis is usually caused by an increase to a certain value of the oxygen unsaturation of the blood (1), the state of affairs described above might be assumed to be due to a decrease in blood flow through the arm as a result of the increased resistance of gravity to venous outflow from the part. If the volume flow of blood through the arm is decreased, after an interval of time, with metabolism progressing normally, a point must arrive where the oxygen unsaturation of the venous blood begins to increase. In the experiments to be presented here, no marked increase in oxygen unsaturation of the venous blood occurred under these conditions. On the contrary, in the majority of cases it was either decreased or it remained the same as the value obtained from blood drawn under conditions where the blue color of the skin was not present.

It is apparent, therefore, that the blueness of the skin observed under the circumstances described is not due to a diminished volume flow of blood in the vessels of the arm, with a consequent increase in the amount of reduced hemoglobin in the blood.

Lundsgaard and Van Slyke (2) conclude that certain factors, although incapable of producing cyanosis themselves, may influence the degree of oxygen unsaturation necessary to impart color to the skin, and modify the intensity of the blue color produced. One which they state ranks high in importance is the number, width and length of blood filled capillaries per unit of skin area.

The results of the experiments in this paper, we believe, show that an engorgement of the vessels whose circulating fluid imparts color to the

[1] A preliminary report of this paper was presented before the Society of Experimental Biology and Medicine, November 19, 1924. (Proc. Soc. Exper. Biol. and Med., 1924, xxii, 87.)

skin may be a cause of cyanosis, in the absence of an increase in the amount of reduced hemoglobin in the blood. The color of the skin may vary, with an unaltered oxygen unsaturation of the hemoglobin. It will be the aim of the authors to elucidate the factors responsible for these phenomena.

Experimental methods. The subject, either seated in a chair or lying upon a couch, allowed the arm to hang down from the shoulder and to remain motionless.

In all of the experiments from which our principal conclusions are drawn, the blood was taken from a vein on the dorsal surface of the hand. Reasons for choosing these veins will be stated later in the paper. Whenever possible, all the samples of blood in a single experiment were taken from the same or, in any event, from a closely adjacent vein.

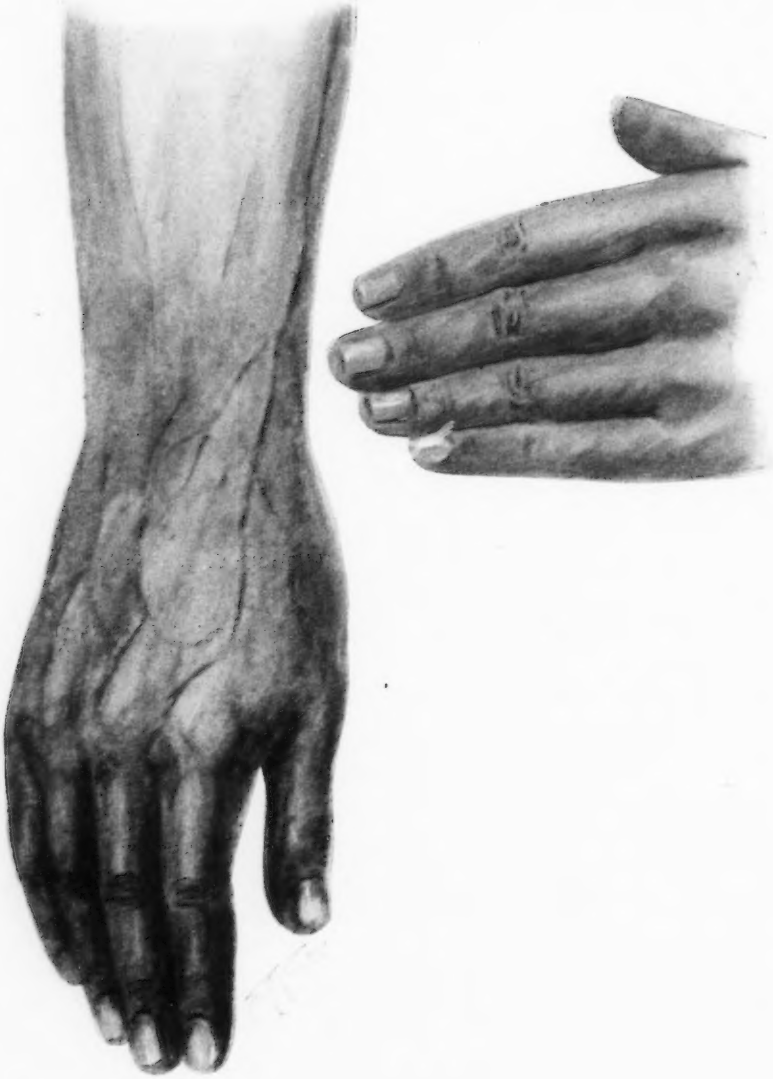
Control samples of blood were obtained with the arm in one of several positions, depending upon the prominence of the hand veins in each subject. The control position of choice was the hand supported at the heart level. It was not always possible to draw blood from the veins in this position for the reason that the vessels were so constricted as to prevent the insertion of a needle. In many instances it became necessary to hang the arm for a brief interval of time until the veins were sufficiently dilated to introduce the needle. This could usually be accomplished within a minute. Another procedure adopted in a few experiments was to hang the arm and puncture the vein. With the needle in place, the subject's forearm and hand were then lifted to the level of the heart by an observer. The blood was allowed to flow continuously from the moment the needle was inserted, to avoid the possibility of a stasis. In a few cases, after the arm had hung down, a second control sample of blood was taken by this last method. With the needle in place the flowing blood was shunted into a second container, while an observer lifted the forearm and hand of the subject to the heart level.

The blood was always drawn without the application of tourniquet, under conditions to insure freedom from exposure to air.

The technique of obtaining the blood and the methods of analysis have already been stated in another paper (3).

Experimental. In table 1 are presented 15 experiments in which comparisons are made of the oxygen content, capacity and unsaturation of the hemoglobin of the blood taken from veins on the dorsal surface of the hand, both before and after the arm had hung vertically from the shoulder for periods of time ranging from 5 minutes to 1½ hours.

A summary of these data showing the deviation of the experimental figures from the controls is set down in table 2. Only experimental figures having controls performed at the same time are thus compared. Of a total of 11 determinations of the oxygen content of the blood, in comparison to the control figures, 6 were increased, 2 decreased and 3



Showing the color of the skin of the hand when held at the level of the heart (horizontal figure) and when allowed to hang down (vertical figure).

unchanged. Differences of a half volume per cent or less are considered as indicating no deviation. Hence in the majority of instances, the oxygen content of the blood was either increased above the control or remained the same, within the limits of error of the methods of analysis.

Eleven figures for the oxygen unsaturation of the blood are partitioned as follows: compared to their controls 3 showed an increase, 5 a decrease, and 3 remained the same. Thus the oxygen unsaturation, under the experimental conditions, was either decreased or remained constant in the greater number of cases.

The oxygen capacity in 13 experiments exhibited an increase from the controls in 3 of the bloods, a decrease in 2, and no change in 8. The rule, therefore, was for the oxygen capacity of the blood to remain unchanged when the arm was thus allowed to hang down.

The carbon dioxide content of the blood in the few cases given (nos. 1 and 2) was decreased with the diminution in oxygen unsaturation.

It is noteworthy that in only one experiment (no. 5) did the oxygen unsaturation of the blood exceed 6 volumes per cent, the supposed capillary threshold for the appearance of cyanosis. Another experiment upon this subject (A. B. L. no. 11) gave an oxygen unsaturation of but 2.5 volumes per cent. In both cases the arm was hung down for approximately the same length of time, and, as far as could be judged, the blue color of the skin was equally intense on the two occasions.

The skin, especially of the hand and wrist, was of a dusky blue color which became progressively less distinguishable above the wrist. In most subjects the blueness developed quite rapidly, and after a short but varying period of time, did not appear to heighten in shade. The depth of the color varied in different individuals. Plate 1 represents an artist's sketch of the hands, one held at the heart level and the other hung down, in experiment 12 (subject S. G.). The appearance of the skin in subsequent experiments (nos. 13, 14 and 15) required no alteration in the picture in order to represent a composite of the condition in the different subjects. Plate 1 shows the striking contrast in the color of the skin of the hand when held at the level of the heart and when held vertically at the side of the body. The resemblance of the latter to a "cyanosis" is unmistakable.

When the arm was hung down, as in the experiments detailed above, there developed rapidly a marked engorgement of the veins of the forearm and hand, also with individual variation.

The results up to this point show that cyanosis may exist under conditions in which the oxygen unsaturation of the venous blood is no greater than in the blood coursing through the normally more constricted vessels.

In experiments 14 and 15 (table 1), after the arm had been lowered for the experimental period and a blood sample drawn, the flow of blood was

TABLE 1

The effect of the posture of the arm upon the gases of blood taken from veins on the dorsal surface of the hand

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	CO_2 CONTENT	REMARKS
		1922	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	
1	And.	August 17	16.00	20.48	78.1	4.48	55.2	Right arm hung down. Blood drawn at once. Subject reclined
			17.78	20.06	88.7	2.28	53.7	Same hand. Arm hung down for 15 minutes
2	Grow	August 18	16.97	19.03	89.2	2.06	50.3	Left hand lowered. Needle inserted. Hand raised level with heart. Subject reclined
			18.51	19.39	95.5	0.88	47.1	Left arm hung down for 18 minutes
3	Seng.	August 18	18.61	20.59	90.4	1.98		Left arm hung down. Blood drawn at once. Subject reclined
			18.12	19.95	90.8	1.83		Left arm down for 15 minutes
4	Ferg.	August 22	18.36	19.80	92.7	1.44	50.1	Left arm hung down. Needle inserted. Hand raised level with heart. Subject reclined
			18.24	19.58	93.1	1.34	50.4	Same arm down for 8 minutes. Blood from same vein
5	A. B. L.	December 14	14.41	21.42	67.2	7.01		Left arm hung down for 1 hour. Subject reclined. Skin dusky blue
				20.73				Arm hung down for 5 minutes
6	A. B. L.	December 18	18.16	20.38	89.2	2.22	47.4	Right arm hung down for 8 minutes
				21.76				Left arm down for 1 hour. Skin blue

7	A. B. L.	December 21	19.49	21.38	91.2	1.89	48.9	Right arm hung down for 18 minutes
			18.19	21.40	85.0	3.21	47.1	Blood from right wrist. Arm down 34 minutes
8	Ad.	December 19	14.44	19.03	75.8	4.59		Right arm hung down 7 minutes. Reclined
			17.56	19.49	90.1	1.93		Left arm hung down 1 hour
9	S. G.	July 21	18.48	20.33	90.9	1.85		Highest of 7 previous determinations on this subject. Arm down 5 to 10 minutes
		December 20	20.25	21.35	94.9	1.10		Left arm hung down for 1½ hours. Dusky blue
		1923						
10	S. G.	January 8	17.23					Right arm down for 20 minutes. Subject reclined. Skin hand blue
			17.16	20.99	81.8	3.83		Left arm down for 9 minutes. Not as blue
			18.21	20.55	88.6	2.34		Right arm down for 25 minutes. Skin blue
		1924						
11	A. B. L.	October 7	20.37*	21.95	92.8	1.58		Right arm hung down, blood drawn at once, 2½ minutes. Subject seated
			21.27	23.80	89.4	2.53		Same arm down 53 minutes. Hand warm and dusky blue
12	S. G.	October 22	19.65	21.18	92.8	1.53		Left arm down, blood drawn at once 1½ minutes. Subject seated
			16.46	20.33	81.0	3.87		Right arm down 38 minutes. Hand warm and dusky
13	S. G.	October 29	18.28	21.17	86.4	2.89		Right arm on level with heart. Subject seated
			18.86	20.85	90.5	1.99		Same arm and vein. Arm down 33 minutes.

TABLE 1—*Concluded*

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	CO_2 CONTENT	REMARKS
		1924	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	
14	D. M. P.	November 7	16.93	20.94	80.8	4.01		Left arm on level with heart. Subject seated
			15.50	20.68	75.0	5.18		Same arm down 30 minutes. Hand warm and dusky
			17.53	20.65	84.9	3.12		Needle in place, arm raised heart level. Blueness disappeared
15	W. W. H.	December 12	17.39	19.26	90.2	1.87		Left arm held heart level. All bloods from same vein
			17.01	18.84	90.3	1.83		Arm down 18 minutes. Dusky blue almost to elbow. Hand warm
			17.46	18.74	93.2	1.28		Needle in place, hand raised to heart level
		Blood from a vein in the antecubital fossa						
		1923						
1E	S. G.	February 5	11.64	21.13	55.1	9.49	56.2	Left arm hung down 18 minutes. Hand dusky blue. Subject seated. Room 19° to 20°C.
			14.79†	21.13	70.0	6.34		Needle in place, hand raised to heart level

* Some air entered the blood after drawing, content therefore was lower.

† Only small amount of blood obtained. One cc. samples for analysis. Used capacity of first blood.

shunted into a second container, while the subject's arm and hand were lifted to the level of the heart. In these experiments, the oxygen unsaturation of the blood was lower than in the control sample drawn with the arm at the chest level, or in the experimental blood obtained after the arm had hung down for some time. A similar experiment in which the blood was drawn from a vein at the bend of elbow, upon raising the arm (1E, table 1) showed the same sharp decrease of oxygen unsaturation. The bearing of these results upon our thesis will be discussed later in this paper.

Inasmuch as the effect produced by hanging the arm resembles, in respect to skin color and venous engorgement, that caused by inhibiting

TABLE 2

Summary of table 1, showing deviations of experimental figures from the controls

NUMBER	VOLUMES PER CENT INCREASE OR DECREASE FROM CONTROL							
	O_2 content		O_2 capacity		O_2 unsaturation		CO_2 content	
	+	−	+	−	+	−	+	−
1	1.78			0.42		2.20		1.5
2	1.54		0.36			1.18		3.2
3		0.49		0.64		0.15		
4		0.12		0.22		0.10	0.3	
5			0.69					
6			1.38					
8	3.12		0.46			2.66		
10	1.05			0.44		1.49		
11	0.90		1.85		0.95			
12		3.19		0.85	2.34			
13	0.58			0.32		0.90		
14		1.43		0.26	1.17			
15		0.38		0.42		0.04		

the outflow of blood from the veins, a comparison of the venous oxygen unsaturation has been made in these two conditions. The results are compiled in table 3. The circulation of the blood in the veins was obstructed by applying to the upper arm and inflating to the desired pressure a Riva-Rocci blood-pressure-cuff. As compared to the data obtained when the arm was hung down, the results with pressure were in striking contrast.

In experiment 1S (table 3) the application for but a few minutes of 60 mm. of mercury pressure produced a venous oxygen unsaturation of 13.91 volumes per cent. For a comparison, the arm was allowed to hang for 1 hour, and in another instance for 53 minutes. The oxygen unsaturation of the venous blood was 7.01 and 2.53 volumes per cent respectively,

though there developed a marked engorgement of the veins with a dusky blue color of the skin of the hand, wrist and lower forearm. The oxygen unsaturation of 7.01 volumes per cent was the highest we ever obtained under the experimental conditions.

TABLE 3

Comparison of the gaseous content of blood from veins on the back of the hand following venous engorgement produced by a constricting band on the upper arm and after hanging and immobilizing the arm

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY Hb.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	REMARKS
		1922	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	
1S	A. B. L.	December 13	10.04	23.95	41.9	13.91	Pressure 60 mm. Hg above right elbow for few minutes. Skin blue
			10.93	22.19	49.3	11.26	Needle still in place, cuff released, arm raised, hand flexed. Blueness, engorgement disappeared
		December 14	14.41	21.42	67.2	7.01	Left arm hung down for 1 hour. Dusky blue
		1924					
		October 7	21.27	23.80	89.4	2.53	Right arm down 53 minutes. Hand warm and dusky blue
		1922					
2S	Ad.	December 19	14.44	19.03	75.8	4.59	Right arm hung down 7 minutes. Subject reclined
			17.56	19.49	90.1	1.93	Left arm down 1 hour
			12.48	19.61	63.6	7.13	Pressure 45 mm. Hg above right elbow for 10 minutes

In a similar trial, no. 2S (table 3), 45 mm. of mercury pressure applied to the upper arm for 10 minutes resulted in a venous oxygen unsaturation of 7.13 volumes per cent, as compared to 4.59 volumes per cent after the arm had hung from the shoulder for 7 minutes, and 1.93 volumes per cent at the end of 1 hour. The increased pressure imposed upon the veins in the two conditions was more nearly comparable in this experiment (2S).

These results show that the oxygen unsaturation of the blood taken

from veins on the back of the hand starts to increase at once (2S) and reaches a high level (1S), when pressure is applied to the forearm for a short time only. The amount of reduced hemoglobin in the blood from veins in the same area, after the arm is hung down for a much longer period, is of a lower order (1S) and, instead of increasing as in the previous case, decreases in amount (2S).

TABLE 4

Comparison of the gaseous content of venous blood from normal and injured cyanotic feet

NUMBER	SUBJECT	DATE	O_2 CONTENT HB.	O_2 CAPACITY HB.	O_2 SATURATION HB.	O_2 UNSATURATION HB.	CO_2 CONTENT	REMARKS
		1922	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	
1P	Gou.	August 16	11.02	20.21	54.5	9.19	54.4	Right foot, normal color. Subject seated, feet down. Blood from ankle vein
			12.47	21.42	58.2	8.95	50.9	Left foot dusky blue, from injury. Seated as above, foot warm
			18.42	19.37	95.1	0.95	47.0	Blood from right hand, hung down 10 minutes. Reclined
		1923						
2P	Mrs. S.	January 11	8.86	16.99	52.2	8.13	60.9	Right foot normal and pale. Subject seated, feet down
			8.00	16.40	48.8	8.40	61.7	Left foot purple, from injury. Subject seated as above

Another difference observed in these experiments is noteworthy. Stoppage of the venous flow by a constricting band produced a cool blue skin; that of the arm hung down was blue but warm.

In the course of this work the opportunity was afforded to investigate two cases of local cyanosis, resulting from an injury to the ankle. The oxygen unsaturation of the venous blood from the cyanotic part was compared to that from the opposite but normal ankle in similar postures. The blood was drawn from the same site and vein on the control and cyanotic foot.

In experiment 1P (table 4), the blood from the normal foot had an oxygen unsaturation of 9.19 volumes per cent, while that from the cyanotic

foot had an unsaturation of 8.95 volumes per cent. The latter sample was drawn from a vein directly in the blue area, and must have drained to an extent the vessels giving the color, provided blood was flowing through them. In this connection it was noted that the injured ankle was warm to the touch, a condition incompatible with a stagnation of blood in the part. Several months had elapsed since the primary injury occurred, hence the acute stage of inflammation had long since subsided.

In the second case (2P, table 4) a similar agreement in venous oxygen unsaturation was found in the blood drawn from the cyanotic and normal area. It is apparent that in this pathological condition the cyanosis was not associated with an increased amount of reduced hemoglobin in the venous blood.

It is of interest to compare the oxygen unsaturation of the venous blood drawn from a normal foot with that from the dorsal surface of the hand in the same individual under comparable external conditions. In the former sample (1P, table 4), a figure of 9.19 volumes per cent was obtained, in the latter 0.95. The factors probably responsible for this difference have already been discussed (3).

The room temperature in the course of the experiments detailed in table 1 varied on different occasions from 21 to 31°C. There was no striking relationship between the external temperature and the effects produced. However, it seemed that in a moderately warm room the blueness developed more quickly than when the room was cool though not cold.

Discussion. The experiments presented in table 1, with one exception (1E), were performed using the blood from veins on the dorsal surface of the hand. There are several reasons for choosing this blood instead of that from veins of the forearm in the antecubital fossa.

The skin of the hand shows a more marked and rapidly developed cyanosis from venous engorgement than does that of the forearm. It is also noticed in general cyanosis that the hand is one of the areas of predilection (2).

Blood taken from superficial veins presents an uncertainty in regard to the proportion of skin and deeper muscular tissue drained by a given vessel. It is believed that blood taken from veins on the dorsal surface of the hand is freer from this objection than that from the upper forearm for the following reasons. The hand is mainly a skin area, with comparatively little muscular tissue. The muscles involved in its flexion are situated above the wrist. The vascular bed for the most part is, moreover, close to the surface, since its principal function is that of warming the part and regulating body temperature (3). A sample of blood taken from a hand vein should then be a nearly uniform mixture, representative of the average venous blood draining chiefly the skin of this member.

Due to the small amount of active muscle tissue, the metabolism in the

hand is probably of a low order, and there is evidence that the blood flow is rapid (3). Consequently, we have found that the average oxygen unsaturation and carbon dioxide content of the venous blood from this area are lower than in the blood taken from veins at the bend of the elbow. In fact, in most individuals it approximates arterial blood in gaseous content (3). It will be noted in this paper that in no case did the normal figure for the oxygen unsaturation exceed 5 volumes per cent; in most of the bloods, it was much less.

There exists, then, a relatively small difference between the oxygen unsaturation of the venous and arterial blood (3). In absolute amount it is much below the threshold (2) of capillary unsaturation believed to be necessary for the appearance of cyanosis. The use of such a blood, therefore, should enable one to confine the threshold value within narrower limits if the occasion warrants. Of even more importance in the present experiments, it allows one to more definitely distinguish factors other than an increased amount of reduced hemoglobin which may be involved in the production of a blue color of the skin.

In spite of the fact that the circulation of blood in the hand is predominantly through skin vessels, there arises the possibility that under the experimental conditions of hanging the arm there may occur a stasis of blood in the capillaries. In such a state, in view of the results reported herein, one must assume that the flow of blood is shunted and maintained through channels other than the superficial vessels. Such a by-pass may be conceived to exist in the direct connections between small arteries and veins alleged to be present in the finger pads of man (4).

In order to test this hypothesis, a few direct observations[2] of the blood flow through the capillaries of the finger at the base of the nail, by the method of Lombard (5), were made both when the hand and arm were hung down from the shoulder and when the hand was on a level with the heart. Upon changing the position of the arm from horizontal to vertical, the venous ends of the capillaries under the nail bed were seen to broaden, an increased number of capillaries were visible in the microscopical field, and the flow of corpuscles through them became more rapid. In addition, the background took on a decided blush, apparently a reflection from the subpapillary vessels. When the arm was again returned to the horizontal position, the reverse of the above picture was seen. The flow of blood continued but was slower, the capillaries became narrower on the venous end of the loop, fewer were distinctly visible, and the background "blush" disappeared. The observations were continued in one case for 15 minutes. Parrisius and Wintterlin (6) observed a somewhat similar phenomenon in the capillaries on the dorsum of the foot. They also examined micro-

[2] We wish to express our gratitude to Dr. J. M. Hayman, Jr., for his assistance in checking these observations.

scopically the finger, and reported that they could observe no difference in the number of capillaries containing static blood, duration of stasis, or speed of flow, when the subject sat with the hand at the heart level, stood with the hand below the heart, or when the arm was raised above the head. These results confirm our findings in indicating that the flow of blood through the capillaries of the fingers is not impeded by the imposition of an increased hydrostatic pressure incident to hanging the arm. Weiss (7) affirmed that a pressure of the order of that normally existing in the arteries must be applied upon the upper arm, before the stream of blood in the capillaries at the base of the nail ceased.

Certain evidence more indirect would also seem to be incompatible with a condition of stasis of blood in the vessels whose contents give color to the skin. As previously stated, the hand which was blue as a result of venous engorgement was nevertheless warm to the touch. Stasis produced by mechanically preventing venous return resulted in a cold skin.

It was noted that when the arm was raised after it had been held down, there was an almost immediate disappearance of the blue color. It is reasonable to expect that if blood were static in some of the skin vessels there would be a tendency for the oxygen unsaturation of the venous blood from the hand to increase with the passage of the stagnant blood from the vessels, coincident with the return of a more normal color. To be sure, the amount of stagnant blood may be but a slight proportion of the total amount collected; nevertheless, a positive outcome of such an experiment would be convincing evidence of a stasis. Such was not the case for, as is shown in experiments 14, 15 and 1E (table 1), there was an immediate decrease in the oxygen unsaturation of the blood flowing from the part. The flow of blood through the needle was not interrupted at any time during this procedure, hence the sample of blood collected should have reflected changes during and after the transition period.

Still another type of experiment was devised to throw some light upon the factor under discussion. If a hand blue from venous engorgement was immersed in water at 5°C., the skin of the immersed part became pink, while that not surrounded by water retained its bluish tinge. When the circulation through the arm was shut off by a constricting band, the cyanosis persisted in spite of exposure to very cold water. The pinkness of the skin as a result of intense cold is, undoubtedly, to be attributed to a depression of the dissociation of oxyhemoglobin and a reduction in the oxidative processes in the cooled tissues (8). For these phenomena to be effective in decreasing the oxygen unsaturation, the blood giving color in the vessels must be flowing. If it were stagnant and the blue color were due to the great increase in reduced hemoglobin content which would accrue therefrom, the blueness would of necessity persist, unless the application of the cold reëstablished the circulation. It is difficult to

ascribe the latter action to cold (8), hence indications are that blood was already flowing through the vessels involved.

The several facts stated above lead us to conclude that the development of the blueness, as a result of hanging the arm in a vertical position, is not due to a stasis and increased oxygen unsaturation of blood in certain superficial vessels with a shunting of the major portion of the flow through other paths.

That the blood drawn from the veins of the hand in this position has not been subjected to an increased deprivation of oxygen, due to a slowing of blood flow, is shown definitely by the evidence presented. In another paper (3) results have been given which indicate that hanging the arm down with the resulting venous engorgement did not increase the average oxygen unsaturation of the blood from veins on the forearm.

There remain, then, two factors to be explained: first, the cause of the cyanosis in the absence of an increased unsaturation of blood; second, the mechanism which compensates for the increase in hydrostatic pressure which produces the venous engorgement, and which, in itself, would result in a slowing of the blood flow through the part.

We shall discuss the latter problem first. Investigators who have made such measurements (9), (10) have found that an increase in hydrostatic pressure incident to change in posture of the limbs raised the venous pressure. There results a visible engorgement of the superficial veins of the forearm and hand. This may possibly be due to the scanty supply of valves in these vessels (11). Also, there is much evidence to show that the capillaries and venules reflect changes in pressure from the venous side of the vascular bed and that consequently they dilate (7), (10), (12), (13).

Since the exchange of gases and fluids between the blood and the tissues takes place in the capillaries and perhaps in the venules, and it is the blood in these vessels which imparts color to the skin (10), (13), they become our primary point of interest in this discussion.

A dilatation of venules and capillaries would increase their capacity and hence their volume of blood available for gaseous exchange. Other factors remaining equal, the rate of flow through the enlarged vessels would be decreased in direct proportion to the increased volume. This would enable the blood to pass into the veins with the same residual load of oxygen as under normal conditions, provided that, when the arm was hung down, the increased hydrostatic pressure which is the cause of the capillary and venule dilatation were in some manner compensated for. The lack of the latter adjustment would inevitably cause a decrease in the volume flow of blood through the arm. This would be evidenced by an increase in the oxygen unsaturation, as in the experiments where the outflow from the veins was inhibited by pressure on the arm (table 3).

Since, for the most part, there is no increase in the oxygen unsaturation when the arm is hung down, it must be concluded that factors of compensation arise.

On a purely physical basis the explanation would appear simple. As the hand was lowered, the rise in hydrostatic pressure on the venous side would be balanced by an equal and opposite pressure on the arterial side of the vascular bed from the same cause. There would thus occur an increase in pressure throughout the whole system, but the two limbs of the U-tube formed by the blood vessels would be in hydrostatic equilibrium. An equally effective mechanism which would at the same time prevent a rise of pressure on the arterial side would be a simple dilatation of arterioles. In either case, after a brief interval during which the distended vessels were filling, the volume flow from the veins would be maintained at a normal rate. Although the volume of blood in the dilated capillaries and venules was increased the velocity of flow would be proportionately decreased, if other factors remained equal. As a result the oxygen passing into the tissues from each unit of blood would thus be constant, assuming that metabolism progressed normally. Hence, the oxygen unsaturation would remain the same.

In this process there may be an additionally effective pressure set free in the capillaries, which would materially aid in overcoming the increased venous pressure. With dilatation of the capillaries and venules, particularly the former, the internal resistance to the passage of blood along these vessels would be decreased. A small increase in arterial pressure, therefore, would result in a relatively larger increase in pressure effective in augmenting venous flow. Theoretically, and in view of recent work, the factor of the decreased resistance to flow in enlarged capillaries would appear of considerable importance. In fact, it may conceivably be the cause of an increase in flow above the normal and thus account for the decrease in oxygen unsaturation observed in the experiments being considered. A measure of the degree of the resistance to blood flow imposed by the capillaries is indicated by the observations of Dale and Richards (14). They found that a dilatation of these vessels might cause a fall in the arterial pressure in the absence of other causal factors.

Mechanisms, such as have been described above, make it clear how one may obtain a constant or even a decreased oxygen unsaturation, in spite of an increased pressure and resulting engorgement in the veins, venules and capillaries due to the heightened hydrostatic pressure imposed upon them by hanging the arm at the side of the body.

The pressure in the dilated capillaries and venules not only would be higher than normal, but their surface area would be increased and they would be under a condition of stretch. This state of affairs, according to the present conception, should result in an increased passage of fluid

through the capillary and venule wall into the tissues. There would be an increased filtration pressure and surface for diffusion, and other factors present, associated with dilatation per se, which Krogh (10, p. 232) states result in increased passage of fluid from the capillaries.

It is of interest to review our findings on the oxygen capacity of the blood, changes in which should reflect alterations in blood concentration. As stated above, in a series of 13 determinations of oxygen capacity, but 3, all on the same subject (A. B. L., nos. 5, 6 and 11, table 1) exhibited a significant increase. Of the remaining 10 figures for oxygen capacity, 2 showed a decrease and 8 remained unchanged.

Inasmuch as the existing conditions are such that an increase in concentration of the blood should occur, and since, in the majority of cases, an increase in the amount of hemoglobin is not demonstrable, the conclusion is obvious that the speed of blood flow through an arm which is hung down is so great that each unit volume of blood is exposed to filtration for a shorter interval of time than normally. As a result, although increased amounts of fluid may pass into the tissues, and in spite of the presence of factors which would inhibit reabsorption of that fluid, the methods at hand fail to detect changes.

It is likely that, when an increased amount of hemoglobin is present, the color produced is intensified (2). In the particular case in which there was a heightened oxygen capacity, such an association was not distinguishable. In fact this subject (A. B. L.) showed a milder degree of blue coloration of the skin when the arm was hung down, than most other subjects investigated.

It has been demonstrated, in table 1, that on raising the arm after it had hung down for a period of time, there resulted a decrease in oxygen unsaturation. The veins collapsed almost immediately and the blueness disappeared rapidly. It is suggested that this phenomenon may be due to a maintenance of an increased blood flow through the hand after the visible collapse of the veins and the vessels whose fluid transmitted the blue color to the skin. That is to say, the veins, venules, and capillaries responded more quickly to the change in position, than did the factors which were the cause of an increase in arterial pressure. This suggests that the latter reaction entails more than a mechanical action of gravity; possibly there is a dilatation of arterioles which persists after the change in the position of the arm.

In discussing the causes of the blue color of the skin of the hand and wrist which appeared when an arm was allowed to hang down, the oxygen unsaturation of the blood, from veins at the back of the hand, will be considered as practically representative of that in the venules and capillaries. This would seem justifiable for several reasons. There is but a relatively slight difference between the oxygen unsaturation of blood from

the veins at the back of the hand and that of arterial blood. An average of fifty determinations of venous blood from this area showed an oxygen unsaturation of 2.4 volumes per cent (3); in many of the controls in table 1 it is even lower than this. These values are much below the threshold of capillary oxygen unsaturation which has been considered necessary for the appearance of cyanosis (2). Furthermore, in cases where the oxygen unsaturation of the venous blood is actually decreased under the experimental conditions, the average capillary unsaturation must vary likewise.

Inasmuch as there is no fixed relationship between the oxygen unsaturation of the blood and the appearance of a blue color of the skin in the experiments in table 1, and since the only other condition known to prevail is a dilatation of venules and at least a portion of the loop of the capillaries, it must be concluded that the color is produced by the latter phenomenon.

Stated more definitely, the vessels whose blood transmits color to the skin, when filled in greater number and increased in width and possibly length, may contain blood of an oxygen unsaturation no greater or even less than that flowing in constricted vessels, yet the skin may be of a bluish hue.

The reasonableness of this assertion may be emphasized in a simple fashion. Although we have found that the blood from veins on the dorsal surface of the hand may have an oxygen unsaturation as low as 0.6 volume per cent (3), this blood viewed in the veins through the skin appears blue. One has only to picture this same blood color diffusely distributed in certain dilated vessels whose contents impart color, to understand the appearance of a cyanotic hue.

In accordance with the general conception of the manner in which skin color is produced (10), all cyanosis of local or general origin must be accompanied by a degree of either capillary or venule dilatation, or both, in order to be visible through the skin. We believe that this factor is not only a necessary accompaniment of all types of skin color but may be a primary cause of cyanosis, in the absence of stasis.

This brings up a point of considerable importance, namely, the cause of differences in skin color under circumstances in which the amount of reduced hemoglobin in the blood is practically the same. In previous experiments (8) we have noted hands pink with cold or heat, when the oxygen unsaturation of the blood from the veins at the back of the hand was scarcely or no greater than in the experiments reported here where the hand was blue. The following explanation is suggested as a cause of this apparent discrepancy.

Spalteholz (15), in drawings founded upon a reconstruction model of the blood vessels of the human skin, showed that the capillaries are confined practically to the papillae. Three venous plexuses are distributed in the cutis and one just below the cutis. The first or subpapillary venous plexus

lies just below the papillae. It is of interest that valves are absent in the cutical veins; it is only in the deepest of the three venous plexuses that they are first noted.

While it is the opinion of some investigators that the color of the skin depends mainly upon the state of dilatation or contraction of the capillaries (10, p. 92), others ascribe this rôle to the venous plexuses (13). From their position as described above, there can be no doubt that both the capillaries and the small veins, which we shall refer to as venules, function in this respect, and under some conditions, the subpapillary plexus of arterioles also.

Since capillaries are closer to the skin, the blood in them would suffer modification in color only in consequence of its passage through the overlying epidermis. The appearance of the blood in the deeper lying subpapillary venules would be altered to a larger degree by the latter factor, since it would have to shine through a greater thickness of tissue, and in addition by its transmission through overlying layers of blood. Hence, under conditions in which both the capillaries and the venules were dilated, the color of the skin would represent the final result of the effect of the venule blood color upon that of the capillary blood, the whole in turn altered in its passage through the overlying tissue. The possibility, therefore, arises that the tint of the skin would differ, depending upon whether the capillary or venule dilatation predominated.

It will be recalled that in a microscopical examination of the vessels of the skin at the base of the finger nail, we noted when the arm was hung down a distinct subpapillary flush, which disappeared on resuming the horizontal position. Changes in color attributable to the subpapillary venous plexus have been observed by other investigators (6), (7), (17), in microscopical examination of the skin. It has also been found that application of pressure on the skin may produce paling, due to emptying of venules, without alteration of the blood content in the capillaries (13), (16).

Applying this conception to the subject at hand, the fact that one may have the same degree of oxygen unsaturation accompanying the pinkness of heat and cold, and the blueness on hanging the arm, may be reconciled as follows. With mild local heat of about 40°C. or slightly above, we have found but small decreases in oxygen unsaturation of the venous blood as compared to that obtained at higher temperatures (8), yet the skin may take on a pink coloration. The capillaries observed under these conditions show a rapid stream in dilated vessels (18). The increased blood flow may be ascribed to a slight relaxation of the arterioles or, as we have suggested above in another connection, to the decreased internal resistance occasioned by the dilatation of the arterial end of the capillary. The color upon the application of mild heat would appear to arise chiefly from the blood in the opened capillaries.

With a degree of local cold which produces a pink color of the skin, the veins and undoubtedly the arterioles are constricted. The dilatation must be in the capillaries and venules. On the assumption that the marked constriction of the veins imposes a heightened resistance to outflow from these vessels, such a dilatation would be explainable. In addition, a weakening of the normal tone of the capillaries by the cold would accentuate the extent to which they would be distended from such a cause. It is probable that the capillaries, under the combined influence of cold and pressure from the venous side, dilate for a further distance towards the arteriole than with increased pressure alone. The skin color, although modified by the blood in the dilated venules, would be reflected principally from the capillaries, as with mild local heat. In this case, any tendency toward an increased blood flow due to reduced internal resistance in the capillaries is offset by the arterial constriction.

The blue color obtained upon hanging the arm is purely a phenomenon of engorgement of venules and capillaries by pressure. In contradistinction to the pinkness of mild heat and extreme cold, the probabilities are that the venule dilatation furnishes the preponderance of the color.

According to this hypothesis, with the same low degree of oxygen unsaturation, predominant dilatation of capillaries would produce a pink color, whereas, the color arising from such a state in the venules would be of a bluish hue. A variety of intermediate shades may be imagined with different degrees of dilatation of the capillaries and venules. For instance, the appearance of the surface is rose-red, often with a purplish hue, upon local application of water at 45 to 47°C. where there is a marked dilatation of all portions of the vascular bed.

It seems hardly necessary to point out that under conditions in which the amount of reduced hemoglobin in the blood is changed, the color transmitted from dilated capillaries and venules will be altered accordingly. The "blueness of cold," which occurs at higher temperatures than the "pinkness of cold" is an example. We believe that when the hand is blue with cold, the dilatation of the skin vessels is produced by an increased resistance to outflow from the contracted veins (8). A coincident contraction of arterioles, however, brings about a slowing of blood flow. This is evidenced by the fact that the oxygen unsaturation of the venous blood is increased. The result is that the blood, darkened in color, must appear bluish through the skin, regardless of whether venules or capillaries are dilated. A predominance of distention of one over the other can only affect the shade.

The main thesis of this paper that dilatation of venules and capillaries may be a primary cause of cyanosis has been found by us to explain the blueness associated with a type of local injury (table 4). It would be of interest to determine whether other pathological conditions exist in which

a local cyanosis may be attributed to this phenomenon. Few data are available at present. One seemingly pertinent example is the polycythemia associated with congential heart disease or Vaquez' disease, where the venous blood may have a normal oxygen unsaturation and contain more oxygen than normal arterial blood (19), (20). An increase in number and width of skin capillaries has been observed in these conditions (20), (7), (17). The color of the skin is usually red, but may become bluish, *particularly in a warm room* (20).

SUMMARY

1. Evidence is presented which shows that when the arm is allowed to hang down and kept stationary the resulting engorgement of the capillaries and the venules of the hand may be a primary cause of blueness of the skin in the absence of an increased oxygen unsaturation of the blood in these vessels.

2. The possible objection that the phenomenon may arise from a stasis of blood in certain superficial capillaries and venules, with a shunting of the blood flow through other channels, is discussed and, we believe, ruled out.

3. The immediate cause of the dilatation of the capillaries and subpapillary venules, when the arm is hung down, is an increased hydrostatic pressure imposed upon the blood in the veins. There results, in consequence, an opening up and engorgement of venules and at least a portion of the loop of the capillaries in the papillae of the skin.

4. The increase in hydrostatic pressure tending to inhibit venous outflow of blood is balanced by a like but opposite pressure on the arterial limb of the vascular bed, thus insuring a maintenance of volume flow of blood through the hand and arm.

5. This effective pressure available for overcoming the increased venous pressure may be augmented to an important degree by the decreased internal resistance to flow of blood through the capillaries, incident to an increase in their cross-sectional dimension.

6. Since the oxygen unsaturation of the blood is still further decreased, upon changing the position of the arm from the downward to the horizontal, in spite of the immediate collapse of veins, venules and capillaries, it is argued that there are present on the arterial side conditions which do not respond immediately to the change in posture. A persisting arteriole dilatation may possibly be the cause of this phenomenon.

7. From the fact that while all the conditions for an increase of fluid passage from the capillaries are present, yet on the whole the oxygen capacity of the venous blood is not increased, it is concluded that the blood flow through the hand is extremely rapid.

8. The suggestion is presented that at a given oxygen unsaturation of the blood, the color transmitted to the skin will depend upon the ratio between the extent of the capillary and the venule dilatation. On the basis of this conception may be reconciled the observations that, at approximately the same degree of blood oxygen unsaturation, the skin is pink when exposed to mild heat or extreme cold while it is blue when the arm is hung down. The blueness of cold and the rose-red or purple of heat are also discussed.

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THE CIRCULATION AND ITS MEASUREMENT

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I. WHAT HARVEY HAS A RIGHT TO EXPECT OF US. In respect to respiration and the circulation, as topics of study and fields for the application of knowledge, physiology offers at present an extraordinary contrast: In one field a mass of exact, quantitative knowledge; in the closely related and equally important department, nothing but conceptions so vague that in practice we scarcely realize that definite quantities exist. In the one a technique, widely used both in laboratory and clinic, to determine exactly the activity of the functions concerned; in the other no accepted method of measurement and, therefore, no estimate of alteration of function more definite than a "considerable increase" or a "slight decrease."

The extent of our knowledge, or rather of our control of these fields, is determined by the effectiveness of the available methods of measurement.

The two fields referred to are those in which the beginnings were made respectively by Lavoisier and by Harvey. Suppose that these two pioneers could come back to survey the development and application of their ideas. Lavoisier would find his main conception fully developed and in active practical use. Every physiological laboratory and every first rate hospital now has apparatus for the determination of basal metabolism, for measurement of the respiratory exchange and the energy expenditure by indirect calorimetry. He would recognize at once such apparatus and methods as the realization of his own plans.

Very different and very disappointing for Harvey would be the contrast in present conditions in respect to the circulation. He would recognize the immense importance of the measurement of arterial pressure and of the evidence that comes from the electrocardiogram, but his interest in these phases of his field would be rather impersonal. We may well imagine him as saying: "Three hundred years have passed since I showed that the blood circulates; twice the length of time that has elapsed since Lavoisier showed that oxygen is consumed. But now when I ask how many ounces or (as you say now) liters of blood are pumped by the heart each minute in this patient, or that athlete, or yonder school child, or exactly how the blood stream varies in rest and exercise, you physiologists cannot answer."

If he asked the clinicians he would probably find only a few who would even understand the bearing of the question. They would in many cases confuse the blood flow with blood volume. So he repeats the question in the form: "What accurate test or measure of the functional efficiency of the heart have you at the beginning of 1925 A.D.?" The answer is: "Virtually none."

The method to be here described aims to fill this lack. If in the hands of others it proves as simple, rapid and accurate as it now seems to us, it will render the circulation as effectively quantitative for clinic and laboratory, in men and animals, healthy and sick, as the respiratory exchange is already. It should make it possible to complete that which Harvey initiated, just as the modern work of Zuntz, Lusk, Benedict, Dubois and others has completed that which Lavoisier initiated.

The lack of a complete working out of the problem of the quantitative circulation has not been due to lack of effort on the part of a long line of investigators of the first ability. For a century the heart was the object of more investigations than any other one organ. Until quite recent times its properties had probably been the subject of more study than any other one topic of corresponding extent in the whole range of science. The volume of the circulation was one of the main topics upon which the great physiological school of Ludwig and his pupils worked. Nearly all of those investigations were, however, carried out under conditions of anesthesia and operation: conditions which are probably as misleading in regard to the circulation as we have learned from Pawlow that they are in regard to the digestive glands, and as Haldane has taught us that they are in respect to the control of respiration. Most of the literature on the volume of the circulation reviewed in our textbooks has, therefore, little bearing on the normal circulation, but is significant chiefly in respect to the processes and degrees of circulatory depression in shock. The entire organism, healthy and under its ordinary living conditions, can alone afford the knowledge that we so much need. If the circulation can be judged from experience with respiration, the best and final evidence must be obtained on man in his ordinary states, sleeping and waking, resting and working, in health and in sickness, old and young, athletic and sedentary.

It is not necessary here to review the extensive literature bearing on the volume of the stroke of the heart and the rate of the blood circulation. A general review (1) of the entire subject was published a couple of years ago by one of us. But the development of thought along the particular line which has led to this paper must be here briefly traced. It was from trails that others had blazed, trails that ended more or less blindly, but which yet led far into the wilderness that our line of search took its origin. It became clear that if the problem is ever to be solved it would require the systematic testing, in respect to certain properties, of all the volatile

substances now afforded by organic chemistry. In this search we found one substance, namely ethyl iodide, whose physical, chemical and physiological properties render it an almost ideal means of measuring the circulation.

The primary purpose of this paper is to describe the method which we have developed for measuring the circulation by means of ethyl iodide, and to report evidence for the reliability and accuracy of that method. Enough evidence regarding the circulation has, however, already accumulated to give a general idea of the results which this method yields. In part they confirm, but in other respects they are quite unlike, what we had expected.

One of us, in the review already referred to, deduced from a survey of the knowledge available two years ago the conclusions: *1*, that the stroke volume of the heart must be for each healthy individual a fairly definite quantity of the order of magnitude of 1.5 to 2.0 ml. per kilo body weight; and *2*, that the stroke volume is subject to variations of only 20 or 30 per cent as between bodily rest and activity. This theory of a fairly uniform stroke volume was directly opposed to the conception of a stroke volume varying by several hundred per cent, as supported by Zuntz a generation ago, and by Starling and his co-workers in recent years. The distinction between these two conceptions lies, not so much in respect to the maximum stroke volume attainable during great exertion, as in the estimate of the size of the stroke during bodily rest. The latter quantity had even been estimated by some writers at only 1.0 ml., or even less, per kilo body weight. The facts as we now find them by means of the ethyl iodide method show that there is for each healthy individual a fairly definite stroke volume of about 1.3 to 1.8 ml. per kilo body weight, and that this amplitude of beat is maintained within moderate limits of variation during rest and in such activities as involve no considerable increase of energy expenditure and breathing. But the effects of posture, lying and standing, in some persons, form an exception to this rule, so that the stroke volume is distinctly larger in the recumbent than in the erect position. In this we confirm the important observations of Bock and Field (2). During vigorous muscular exertion also the stroke volume may be increased a hundred per cent, reaching 3 ml. or even a little more per kilo per beat. But the fact that the normal resting stroke volume is much larger than was formerly believed eliminates such an extreme variability as was then inferred, and indicates that stroke volumes of the order of magnitude of 0.5 ml. per kilo occur only as concomitants of hemorrhage, shock and other conditions of depressed vitality.

The meaning of the larger circulation thus evidenced both for bodily rest and during muscular work is clear and important. It is that in healthy, vigorous persons the circulation performs its respiratory function

to a remarkably high efficiency. During rest the circulation is so large in relation to the oxygen consumption of the body that the difference in the oxygen content of venous and arterial blood is only 3 or 4 volumes per cent, an oxygen utilization of only 20 per cent. During work corresponding to a seven- or eight-fold increase of energy expenditure and respiratory metabolism the arterio-venous oxygen difference rises to only about 8 volumes per cent or 40 per cent utilization. These figures, when applied to the oxyhemoglobin dissociation curve at appropriate pressures of CO_2 indicate that during rest the average pressure of oxygen in the blood as it leaves the tissue capillaries is as high as 50 mm., and that even during vigorous sustained exertion it does not fall appreciably below 40 mm. With a slower circulation, and consequently a higher percentage of utilization of the oxygen from the blood, as after hemorrhage and other conditions of depression, the pressure would be correspondingly decreased. As this pressure is the force which drives oxygen from the blood in the capillaries into the tissues, its amount is one of the fundamental conditions determining the power attainable in the ultimate working elements of the body, the living cells.

But it is noteworthy also that this advantage of a low arterio-venous oxygen difference, and the consequent high capillary oxygen pressure, are gained at a cost in work by the heart, which is much higher than has heretofore been generally estimated. The results here to be reported show that in a man of 70 kilos each ventricle of the heart may discharge as much as a 0.25 liter at a beat, or 0.5 liter for the two ventricles, and that during bodily work, such as riding a bicycle against a resistance, even when the work falls considerably short of maximum exertion, the volume of blood that flows through the lungs may reach 30 liters or more per minute.

The circulation and respiration are the two halves of a single process: the maintenance of the interior atmosphere.

II. Application of Fick method to man. One of the classic lines of attack on this problem is by means of the so-called Fick method. Many investigators in recent years have attempted to adapt this method to the determination of the circulation in man.

As applied to animals, the principle of the Fick method is simple, although laborious and limited to operative, or at least to quiescent, conditions. In essentials the procedure is as follows: Blood is obtained from both the right and left sides of the heart or, instead of the left heart, from an artery. At the same time the volume of air breathed per minute is measured, and a sample of the expired air is analyzed to determine its gain of CO_2 and its loss of oxygen. Suppose that the analyses of the blood show that the venous blood contains 4.0 volumes per cent more CO_2, and 5.0 volumes per cent less oxygen, than the arterial. Suppose also that, from the measurement of respiration and from the analysis of the expired

air, it appears that 0.04 liter of CO_2 are being ventilated out of the blood and exhaled each minute; and that 0.05 liter of oxygen are being absorbed. Evidently the CO_2 elimination divided by the CO_2 differential between venous and arterial blood gives us the blood flow through the lungs. Similarly the oxygen consumption divided by the oxygen differential between the arterial and venous blood gives the same figure. Both show a circulation of 1.0 liter per minute. This reasoning reduces to the formulae

$$\frac{CO_2 \text{ Output}}{\text{Venous } CO_2 - \text{Arterial } CO_2} = \text{Circulation}$$

$$\frac{O_2 \text{ Consumption}}{\text{Arterial } O_2 - \text{Venous } O_2} = \text{Circulation}$$

In the various attempts to modify this method for application to man, the impossibility of obtaining the mixed venous blood has been the obstacle to be circumvented, since it cannot be directly overcome; for blood from a peripheral vein will not serve, but only the mixed venous blood from the right heart. The oxygen and the CO_2 dissociation curves of the blood are, however, known or can be obtained. Accordingly, the content of both oxygen and CO_2 in the arterial blood can be fixed with a fair degree of accuracy by applying to these curves the partial pressures of CO_2 and oxygen in a sample of air from the depths of the lungs. The figures for these pressures are usually obtained by the method of Haldane and Priestley by a deep expiration through a tube and analysis of a sample of air from the nearer end of the tube.

For the venous gas tensions a gas mixture must be found which is unaltered when breathed back and forth between a bag and the lungs for a few seconds. For this purpose series of artificially prepared mixtures of gases were used by Douglas and Haldane (3), who thus tried to find the partial pressures of oxygen and CO_2 that would be in equilibrium with the venous blood. By applying these tensions to the dissociation curve of the venous blood they derived the gas contents. This method is, however, so laborious as to invalidate, we believe, not only its practicality but to some extent also its reliability. The blood, as Haldane and his co-workers have shown, does not take up oxygen and CO_2 independently of each other, but each gas tends to expel the other. This fact, which is taken into account in their procedure, complicates the problem and renders the results correspondingly uncertain. To meet this difficulty in another way Burwell and Robinson (4) have carried out an equilibration of blood samples with the gas mixture tested in the lungs, but this procedure involves a succession of gas and blood analyses whose errors may be summated in the results.

A much simpler procedure, and one which because it is simpler is, we believe, more reliable, is that suggested by Henderson and Prince (5) for

obtaining "virtual venous air." It aims only to determine the tension of CO_2 in the venous blood after the blood has been fully oxygenated without loss of CO_2. The value so obtained is applied to the CO_2 dissociation curve of blood which is fully oxygenated. Improvements in this procedure have been made by Meakins and Davis (6) and by Field, Bock, Gildea and Lathrop (7). As it now stands it is as follows:

The apparatus used for this method we call a "tensimeter." It is shown in figure 1. It is essentially a four-way stopcock so arranged that the mouth can be connected in rapid succession to an opening to room air, to a Haldane tube, and to a Plesch bag. It consists of a slide valve formed by two round pieces of sheet brass (9 cm. diameter and 0.25 cm. thick) ground and greased so that one piece turns on a bolt in their common center and slides over the other without leakage. There is a single hole

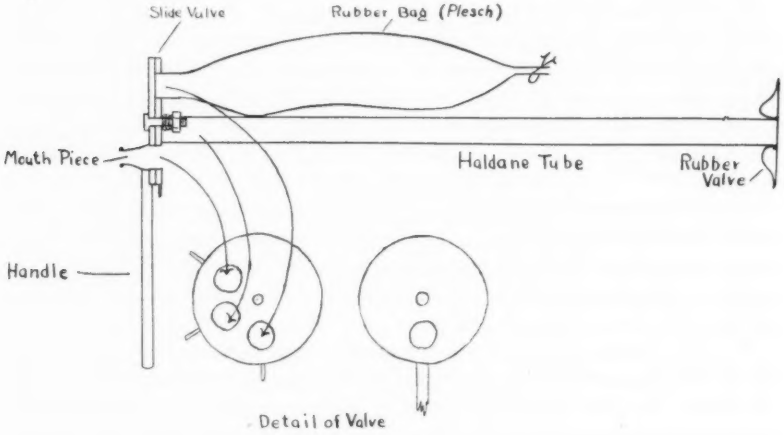

Fig. 1

in one plate to which is attached a mouthpiece, while in the other plate there are three holes, each of which can be turned in succession to lie in front of the mouthpiece. One of these three holes opens merely to the air; to the second is fastened a tube 2 cm. in diameter and 60 cm. long with a rubber valve at its far end; to the third hole is fastened, by means of a short piece of copper tubing, a 3-liter rubber bag.

The bag is filled with oxygen containing 5, or even better 6, per cent CO_2. (This mixture is now extensively used for the treatment of carbon monoxide asphyxia, and is obtainable in cylinders of compressed gas.) The subject then breathes as naturally as possible through the mouthpiece with the apparatus turned so that he inhales and exhales room air. Then the movable plate is turned so that the tube is brought in front of the mouthpiece, and the subject makes a very deep expiration. Immediately the movable plate is turned further, so that the air in the tube is trapped, and that the rubber bag is brought in front of the mouthpiece. The subject then inhales and exhales as deeply as possible from and into the bag four or five times during ten or fifteen seconds. The procedure ends with a deep expiration into the bag, and the plate is turned to trap this gas in the bag.

A sample of the air in the tube from the initial deep expiration is drawn off through a small side opening, and is analyzed for CO_2. It gives the CO_2 pressure in the alveolar or "arterial pulmonary" air. A sample from the bag is also analyzed for CO_2. The whole procedure is then repeated until two successive analyses of the CO_2 in the bag agree within about 0.1 per cent. The figure so obtained is taken as the "virtual venous" CO_2 tension.

The arterial and venous tensions, obtained as here described, may be calculated in millimeters and these figures may be applied as abscissae to the CO_2 dissociation curve of fully oxygenated blood. From the corresponding ordinates of these points on the curve the CO_2 contents, expressed in volumes per cent, are obtained. An empirical equation for the CO_2 dissociation curve of fully oxygenated blood (8) is:

$$y = -0.0035x^2 + 0.775x + 26.2$$

But in practice it is sufficient to regard the curve in the physiologically important region as a straight line, and to assume that by multiplying the difference in the percentages of CO_2 in the arterial and virtual venous airs by the factor 3.33, the difference between the arterial and venous CO_2 contents is obtained. Thus if the tensions of CO_2 in the alveolar air, a, and virtual venous air, v, are expressed in per cent of an atmosphere, dry, just as the analyses give them; and the difference, d, between the CO_2 of arterial and venous blood is expressed in volumes per cent, the formula is:

$$3.3(v - a) = d$$

The difference, d, divided into the CO_2 that the individual is exhaling each minute gives the number of liters of blood flowing through the lungs per minute, in other words, the circulation. An example of such a determination is as follows:

Subject Y. H. Weight 80 kilos. Sitting rest, 2 hours after light breakfast. Pulse 64. Alveolar CO_2, 5.2 per cent. Virtual venous CO_2, 6.4 per cent. Difference, 1.2 per cent. This difference multiplied by the factor 3.33 gives a difference of 4.0 volumes per cent CO_2. Output of CO_2, 0.280 liter per minute. Output divided by CO_2 difference gives the circulation as 7.0 liters per minute.

Among the disadvantages of this method is the fact that it requires the voluntary coöperation of the subject, involving training him to this extent in respiratory methods or, as it has been significantly termed, "respiratory gymnastics." Another disadvantage is that it holds good only so long as the blood has a normal CO_2 dissociation curve. It can be applied to abnormal conditions, or to a healthy subject during exercise, only by obtaining arterial blood, analyzing a portion of it, and equilibrating the remainder with the virtual venous gases: a complicated procedure to which the objections have been pointed out above. In our experience the results even on

a single trained individual vary about their mean value much more widely than do the determinations with the ethyl iodide method.

In its simpler form, however, this method is valuable. It is very instructive for teaching purposes. In the hands of Bock and his associates it has also produced valuable information regarding the remarkable influence which posture may have upon the stroke volume of the heart. Recently Doctors Bock and Field visited this laboratory and gave us the benefit of a comparison of the results which they had obtained by the method of the virtual venous air with those which we obtain by the ethyl iodide method. The latter seems to give somewhat the higher figures for the circulation. But on the whole the results by the two methods are so clearly in agreement that they afford definite evidence for the general reliability of the ethyl iodide method.

III. Measurement of the circulation with foreign gases. For the purpose of measuring the blood flow through the lungs in man, it would evidently be advisable to employ some gas whose solubility in blood would be constant under all bodily conditions, health and disease, rest and exercise; in contrast to the variability which blood exhibits in its capacity for CO_2 and oxygen.

Nitrogen. The use of the nitrogen dissolved in the blood was tried by Bornstein (9). He attempted to determine the rate at which this dissolved nitrogen is eliminated when oxygen is breathed. Of this general idea it may be said that, if there were a good direct method of analyzing for gaseous nitrogen, this line of attack would offer possibilities, although the solubility of nitrogen is inconveniently low. Actually the use of nitrogen to this end has not been found practicable.

Nitrous oxide. The most serious attempt along this line has been that of Krogh and Lindhard (10) with nitrous oxide. For practical purposes this effort also has failed, but it was a valuable step in development, and the reasons for its failure in practice are instructive. From a bag or small spirometer a convenient percentage of nitrous oxide in air or oxygen is inhaled. It was thought necessary that the whole procedure should be completed before sufficient time elapsed for any blood, which has once been exposed to nitrous oxide in the lungs, to complete the round of the circulation and come back to the lungs from the venous side. As this period even during bodily rest is only 30 or 40 seconds, it does not allow sufficient time for the various necessary steps. These steps are: *1*, breathing deeply back and forth into the bag, sufficiently often (at least three times are necessary) to give complete mixture of the gas in the bag with the air in the lungs; *2*, a half expiration to afford an alveolar sample (but half expiration can not be relied on to give an alveolar sample, see review (1)); *3*, holding the breath for 10 or 15 seconds to allow absorption of nitrous oxide into the blood going through the lungs; and *4*, a deep expira-

tion from which a second alveolar sample and estimation of the amount of nitrous oxide absorbed into the blood are obtained.

Fifteen years ago in connection with the Pike's Peak Expedition, the use of nitrous oxide for the measurement of the circulation was tried in this laboratory; but also without success.

On the whole, it seemed to us that so short a period of inhalation as that used in the method of Krogh and Lindhard cannot afford reliable results; and in fact all the data obtained by that method exhibit wide experimental variations. We therefore decided to face the error of double or even triple or more circulations of the blood, for the sake of better measurements on other points. We thought it might be possible in calculating results to correct for the error involved in the return of a considerable amount of a gas in the venous blood. At about the time that we reached this conclusion the subject of the absorption and elimination of ether was being investigated by one of us. We attempted, therefore, to utilize ether for measuring the circulation.

Ethyl ether. In principle the calculation of the circulation along the proposed lines depends upon determining two quantities; *1*, the amount absorbed per minute, and *2*, the concentration in the arterial blood with a correction, if needed, for the amount returning in the venous blood. The first of these factors is then divided by the second, and the quotient expresses the volume of blood passing through the lungs per minute. By the use of the iodine pentoxide method of analysis it is easy to determine with a high degree of precision the concentration of ether in the air inspired and in that expired. The difference between these two quantities is then multiplied by the volume of the air breathed, as measured by means of a a spirometer; and the product affords a figure for the amount of ether absorbed per minute.

Difficulty arises, however, when we attempt to estimate the concentration of a substance like ethyl ether in the arterial blood without employing arterial puncture. If alveolar air is obtained and analyzed, the concentration of ether found in it must be multiplied by the coefficient of distribution, which for ether is 15, in order to get the figure for the concentration in the arterial blood. A small deviation in the figure for the alveolar concentration of so soluble a substance as ether makes, therefore, fifteen times as great a difference in the figure derived for the arterial blood, and a correspondingly great variation in the calculated volume of the circulation. The rapidity of absorption of ether is such that a correct alveolar sample can scarcely be obtained by the method of Haldane and Priestley; and an indirect calculation from the dead space was therefore resorted to. Again owing to the high figure for the coefficient of distribution of ether, the addition or subtraction of even a few hundredths to the value assigned to the dead space causes an enormous variation in the figure calculated for the circulation.

The sum and substance of our rather extensive tests of ether as an agent for measuring the circulation in animals and men is that its concentration in the arterial blood cannot be estimated sufficiently accurately by merely respiratory methods. The coefficient of distribution is too high to allow a sufficiently close estimate of the alveolar concentration as calculated from the dead space. On the other hand, the withdrawal of arterial blood and direct analysis of its content of ether make it possible to measure the circulation effectively and accurately. The following experiments illustrate the inherent inaccuracy of measuring the circulation in man with ether, but without arterial puncture. They show, however, that quite accurate results are obtainable on animals, and probably also therefore on man, by means of ether vapor when arterial blood is used. Convenient quantities for use on man would be about 5 mgm. of ether per liter of inspired air, and 10 ml. of arterial blood for analysis in the iodine pentoxide train.

Calculation of circulation in man by means of ether without arterial puncture. Note how widely the figures for the circulation are perverted by a slight difference in the figure used for the dead space.

Subject Y. H., 80 kilos; standing, pulse 70. Respiration, 10 liters per minute. Inspired air, I, contained 10 mgm. ether per liter. Expired air, E, at end of 2 minutes, contained 3.3 mgm. ether per liter. Amount of ether absorbed per minute, 67 mgm.

	(1)	(2)
Assuming dead space, D, at two slightly different values..	0.32	0.30
The corresponding alveolar concentrations, A, become, by the formula $\frac{E-(I\times D)}{1-D}$	0.147	0.428
Coefficient of distribution.....	15.0	15.0
Circulation, liters per minute $\left(\frac{67}{A\times 15}\right)$	30.4	10.4

Experiments illustrating measurement of circulation in dogs by means of ethyl ether; all samples taken at the end of the first minute of inhalation

RESPIRATION PER MINUTE	CONCENTRATIONS PER LITER OF AIR		AMOUNT ABSORBED	CONCENTRATION PER LITER OF BLOOD		CIRCULATION PER MINUTE
	Inspired	Expired		Arterial blood	Venous blood	
liters	*gram*		*gram*	*gram*		*liter*
1.6	0.2	0.061	0.222	0.32	0	0.612
2.1	0.5	0.171	0.691	0.74	0	0.933

Hydrogen. With hydrogen, owing to its very low solubility, the difficulties are of a different character. The difference between the inspired and expired concentrations is so small as to be difficult to determine with a sufficient degree of accuracy. Such variations affect the estimation of the circulation almost proportionately. Another difficulty is as follows: Whereas with a highly soluble gas like ether, a relatively long time is

required to reach saturation, for the capacity of the body is so large that the substance virtually disappears as the blood passes through the tissues during the first few minutes that the vapor is breathed; on the other hand with a gas which is so slightly soluble as hydrogen, the capacity of the body to hold any considerable quantity is small, and the rate of saturation is therefore rapid. The following examples illustrate why hydrogen is too slightly soluble to be easily used for measuring the circulation.

Calculations illustrating inaccuracy of measurement of circulation by means of hydrogen

	I	II
Respiration, liters per minute	7.0	7.0
Hydrogen in inspired air, per cent	3.00	3.00
Hydrogen in expired air, per cent	2.94	2.96
Hydrogen absorbed, per cent	0.06	0.04
Hydrogen absorbed, milliliters per minute (m)	4.2	2.8
Hydrogen in alveolar air, calculated on assumption that dead space is 33.3 per cent	2.91	2.94
Coefficient of distribution	0.018	0.018
Hydrogen in arterial blood, milliliters per liter of blood (n)	0.524	0.529
Circulation, liters per minute $\frac{(m)}{(n)}$	8.0	5.3

Ethylene. As ethylene is now rather widely used as an anesthetic, a series of experiments was performed to test its possibilities for measuring the circulation. Unfortunately the coefficient of distribution is not exactly known; and although we determined it, we were later led to doubt the strict accuracy of the determination. In general ethylene has convenient values, as the following example from among our experiments illustrates:

Type of experiments with ethylene.

Subject Y. H. Weight 80 kilos. Standing, pulse 70. Respiration 10 liters per minute. Inspired air contained 1.0 mgm. of ethylene per liter. Expired air was collected in 5 Douglas bags successively, for 2 minutes in each bag; and analyzed for ethylene by means of the iodine pentoxide train. Bag I, 0.86 mgm. ethylene per liter of air; II, 0.82; III, 0.82; IV, 87; V, 92. Using the figures from bags II and III and the respiration, we have 1.8 mgm. ethylene absorbed per minute. Assuming a dead space of 33 per cent, we have $\left(\frac{0.82 - 0.33}{1 - 0.33} = 0.73\right)$ the alveolar concentration 0.73 mgm. With a coefficient of distribution of 0.2 the arterial concentration comes to 0.146 mgm. Now dividing 1.8 by 0.146 the circulation is calculated as 12.3 liters per minute.

Many other substances also were considered, or tested, but rejected on one ground or another. Thus chloroform is not analyzable by means of iodine pentoxide; ethyl chloride is too soluble in water; and so on. Thus in respect to carbon monoxide, a little consideration shows that the same

properties which fit that gas to serve as means of measuring the blood volume, render it impossible as a means of measuring the blood flow. Simultaneously with these studies an investigation was carried out by one of us as to the principles underlying the absorption and elimination of ether (11), in order to afford a more precise scientific basis than has heretofore existed for the induction and termination of anesthesia. These principles will now be formulated here as they apply to the absorption, distribution and elimination of non-reacting gases and vapors in general.

IV. Absorption, distribution and elimination of non-reacting gases. By a non-reacting gas is meant one which is taken up, held and given off again by the blood and the tissue fluids of the body in simple solution; and which undergoes no destruction or alteration in the body, but is both absorbed and eliminated simply by diffusion from and into the air of the lungs and into the urine. It may or may not have pharmacological action. Examples of such gases are nitrogen, methane and ethyl ether. The first has been studied especially by Haldane (12) in relation to caisson disease; the last recently by Haggard for anesthesia. The absorption and elimination of such gases depend upon four main factors.

1. The rate at which a gas is absorbed into the body, and the amount the body can hold, vary greatly according as the gas is more or less soluble. In general the solubility of a gas in blood is only slightly less than its solubility in water. It is essential that the solubility should be determined at body temperature. The capacity of the various tissues to dissolve gases varies widely; it is low in bone and often very high in fat; but with most gases the average for the body as a whole is probably nearly the same as the solubility in blood. The total amount of any gas that the body will take up is dependent both upon its solubility, and upon the concentration in the air breathed.

2. The rate of absorption is directly proportional to the concentration of the gas in the air breathed. The concentration in the air is generally best expressed in milligrams of the gas per liter of air. The concentration that is effective is, however, not exactly the concentration in the air of the room; it is the concentration when the air is warmed to body temperature and saturated with moisture. It is the somewhat reduced concentration under these conditions, as they obtain in the lungs, which induces diffusion into the blood passing through the lungs. At the saturation point, the total amount of the gas in the body is directly proportional to the concentration in the atmosphere, with which the blood and body as a whole have come into equilibrium. From twice as high a concentration the body will absorb twice as much of the gas.

3. The third factor determining the rate of absorption is the pulmonary ventilation. It depends upon the volume of air breathed, and the amount of the gas thus brought into contact with the blood in the lungs, but not

that which merely enters the dead space. As we shall see, respiration is particularly important in respect both to absorption and elimination in the case of quite soluble gases, such as ethyl ether; but it is comparatively unimportant in respect to relatively insoluble gases, such as nitrogen.

4. The circulation is the fourth factor, and is largely determinative of the rate of absorption and elimination of gases of low solubility, while for such gases respiration plays a comparatively small part. Thus a condition, which would double the volume of air breathed per minute, would only slightly affect the entrance or exit of such a gas, so long as the circulation remains constant. But if the circulation were doubled, so that twice as much blood would flow through the lungs each minute, even with respiration unchanged, the rate of absorption and elimination of such a gas would be nearly doubled.

Evidently for the purpose of measuring the circulation we need a gas of rather low, but not too low, solubility.

The factors concerned in these processes and conditions may be conveniently expressed by such symbols and formulae as the following:

L, effective pulmonary ventilation in liters per minute, that is, respiration minus the volume of air which merely enters the dead space.

C, concentration of the gas in milligrams per liter of inspired air, when warmed to body temperature, and saturated with moisture, as it is in the lungs.

K, coefficient of distribution of the gas between equal volumes of lung air and pulmonary blood. For ordinary conditions complete and instantaneous equilibrium between arterial blood and lung air may be safely assumed. Thus if for a certain gas, such as ethyl ether, K is 15, this figure means that there is always in the blood leaving the lungs, and in the arterial blood, fifteen times as much of that gas in one liter of blood as in one liter of alveolar air; and that this holds true alike during absorption, equilibrium and elimination of the gas.

G, liters or kilos of blood in active circulation in the body. In other words, the effective blood volume at the time.

B, circulation, defined as the number of liters or kilos of blood flowing through the lungs in one minute.

A_c, concentration of the gas in the arterial blood in milligrams per liter.

V_c, concentration of the gas in the mixed venous blood in the right heart in milligrams per liter.

W, body weight in kilos.

The orders of magnitude indicated by these symbols for a healthy adult at rest are approximately as follows: L, 5 liters per minute, corresponding to a respiration of 7.5 liters and a dead space of one-third. W, 70 kilos. G, 5 to 7 liters. B, 1.3 to 1.8 ml. per kilo body weight per heart beat, or

6 to 9 liters per minute. K, for nitrogen 0.014, and for ethyl ether 15.0. For orientation in this field it may be added that for the anesthetic tension of ethyl ether, C is about 100 milligrams per liter, and that for the nitrogen in the air in the lungs (i.e., at body temperature saturated with water vapor) at atmospheric pressure at sea level C is 723 milligrams per liter. In a caisson at 15 pounds gage pressure, that is one atmosphere additional pressure, it would be 1492 milligrams.

The conditions determining the absorption and elimination of any nonreactive gas may be defined in terms of these symbols by the following expressions:

(1) LC = amount of the gas inhaled into the lungs per minute.

(2) $LC \times \frac{L}{BK + L}$ = amount of the gas again exhaled from the lungs per minute.

(3) $LC \times \frac{BK}{BK + L}$ = the amount of the gas absorbed per minute into the blood, before any of the blood completes the round of the circulation, and returns to the lungs from the venous side; that is, the initial rate of absorption.

(4) $\frac{LCK}{BK + L}$ = amount of gas in milligrams absorbed per liter of blood flowing through the lungs.

(5) $\frac{LCKG}{BK + L}$ = amount of gas absorbed during the first complete circulation of the blood, which is accomplished in the time $\frac{G}{B}$, a period less than one minute even during bodily rest.

(6) $\frac{LCKG}{BK + L} \times \frac{G}{W}$ = amount of the gas brought back to the lungs by the venous blood during the second round of the circulation.

This amount is added to the gas which the breathing brings to the lungs; and the sum of the two quantities is distributed between the arterial blood and the expired air in accord with the volumes of blood and air and as determined by the coefficient of solubility, so that the second round of the circulation carries

(7) $\left(\frac{LCG}{B} + \frac{LCKG^2}{W(BK + L)}\right) \times \frac{BK}{BK + L}$ = amount of gas carried in arterial blood from lungs to tissue capillaries during second round of circulation.

This merely means that the sum of the gas inhaled, and that brought back to the lungs in the venous blood, is always distributed between the arterial blood and the expired air in the ratio $BK:L$. It means also that the mixed venous blood flowing back from the body always contains an amount of the gas per liter, which is the same as the average concentration (in milligrams of the gas per kilo body weight) in the body as a whole at the moment.

These expressions signify also that during absorption the arterial blood contains at first far more of the gas than the venous blood. But as the inhalation of a constant concentration of the gas in the air continues, the concentrations in the arterial and venous bloods gradually approach the same value, namely, that of saturation for the concentration of the gas in the air. During elimination the venous blood has a concentration which is the average of the concentration in the body as a whole, while that of the arterial blood is lower than the venous by the fraction thrown off in the breath.

(8) $V_c\left(1 - \frac{L}{BK + L}\right) = A_c$, the arterial concentration during elimination.

The amount of any gas that the body will hold is defined by the expression:

(9) CKW = amount of gas in body at equilibrium with C in the air.

Evidently the maximum amount of a gas that can be absorbed is not a fixed absolute quantity, but is proportional to the concentration of that particular gas in the air. When respiration and circulation remain uniform and normal, the rate of absorption is such that, if a certain percentage of saturation is taken up in one minute, the same percentage of the remaining unsaturation will be absorbed in the second minute, and so on thereafter. Thus, if saturation be taken as 100, and if one per cent of this amount is absorbed in one minute, one per cent of 99 will be absorbed in the second minute; and one per cent of 98.01 in the third minute, and so on. The absorption is thus comparatively rapid at first, then slower and finally infinitely slow. It is not practical, therefore, to determine the time when equilibrium of intake and elimination will be reached. But it is often convenient for purposes of calculation to use the time required to reach 50 per cent saturation, $\frac{CKW}{2}$, or some other percentage of saturation.

The time required to reach a certain percentage saturation x, may be derived as follows. Let A be the amount absorbed in the time t; then since CKW is the maximum, or limiting amount, that can be absorbed and since

$$\frac{dA}{dt} \propto (CKW - A); \text{ therefore } \frac{dA}{dt} = k(CKW - A)$$

Integrating with θ as the constant of integration;

$$\ln [\theta\ (CKW - A)] = -kt, \text{ and } \theta\ (CKW - A) = e^{-kt}.$$

$$A = CKW - \frac{e^{-kt}}{\theta}, \text{ but when } t = 0,\ A = 0, \text{ and therefore } \theta = \frac{1}{CKW}.$$

Accordingly

(12) $A = CKW\ (1 - e^{-kt})$

Now if x equals the percentage of saturation reached in time tx,

$$x.CKW = CKW.\ (1-e^{-kt}x).$$

$$\frac{1}{1-x} = e^{-kt}x, \text{ or } kt_x, = \ln \frac{1}{1-x}$$

$$(13)\ t_x = \frac{2.3}{k} \log \frac{1}{1-x}$$

Since at the start $A = 0$, therefore, $\frac{dA}{dt} = k\,(CKW)$; and from general consideration initially $\frac{dA}{dt} = \frac{LCKB}{BK+L'}$, combining these two expressions we have $k = \frac{LB}{(BK+L)W'}$, which can be substituted in (12) and (13).

The use of the half saturation time, or the time of any definite percentage of saturation, is particularly convenient because it is the same for all concentrations of any one gas, so long as respiration and circulation are uniform. But the greater the solubility of a gas, the longer is the time required to reach half saturation, and the greater the absolute amount constituting half saturation.

For an average human adult at rest the time of half saturation for a gas of $K=15$, such as ethyl ether, is about 2.5 hours, while for one of $K=0.014$, nitrogen, it is about 7 minutes, and for 90 per cent of saturation about 22 minutes. For a child, owing to the more active metabolism and greater relative volume of breathing and circulation in relation to body weight, the time required to reach half saturation, or any other definite percentage, is correspondingly less. The same relation holds true in an adult, when respiration and circulation are increased by exercise, or by the pharmacological action of the gas itself, or in any other way.

The absorption of any absolute amount of gas, for example the anesthetic amount of ether, is effected very slowly, when a low concentration of the gas is inhaled; but with a far more than proportional rapidity, when the concentration in the air is high. The rates of absorption are not expressed by straight lines, but by exponential curves, which rise rapidly at first and then more and more slowly. In the absorption curves of all concentrations of a given gas, points having equal abscissae have ordinates which are proportional to the concentrations. An absolute amount in milliliters may, therefore, be only a relatively low percentage of the saturation limit (CKW) of a high concentration, but a high percentage of the limit, or even exceed the limit, for a low concentration.

The influence of the respiration, the circulation, and the solubility of the gas upon the time required to reach 50, or any other, per cent of saturation, is expressed by the formula:

$$\frac{t_x}{t'_x} = \frac{\frac{BK+L}{LB}}{\frac{B'K'+L}{L'B'}}$$

If we consider a condition such as that of bodily rest, in which the circulation and respiration remain constant, we may set B and B', L and L', as all equal to each other and simplify the fraction accordingly. If we consider the effect of the solubility of gases under these conditions, and compare the length of time necessary to attain half saturation with a gas like ethyl ether, which has a high coefficient of distribution, and another gas like nitrogen, which has a low coefficient of distribution, we see that the time required in the former case will be the greater almost in proportion as K is greater than K', thus:

$$\frac{t_x}{t'_x} = \frac{K + 1}{K' + 1}$$

If we consider the condition in which respiration is constant, but the circulation is varied, that is, L equals L', while B is greater than B', we find that the length of time required to reach half saturation is greatly shortened by an increase of the circulation in the case of a comparatively slightly soluble gas; but is very little affected by the rate of the circulation in the case of a comparatively soluble gas. Thus

$$\frac{t_x}{t'_x} = \frac{\frac{BK + L}{B}}{\frac{B'K' + L}{B'}}$$

Finally, if we consider the condition in which the circulation is constant, but the respiration is varied, that is, B equals B', while L is greater than L', we find that the length of time required to reach half saturation is greatly shortened by an increase of respiration in the case of a very soluble gas; but is very little affected by the rate of respiration in the case of a comparatively insoluble gas.

Thus

$$\frac{t_x}{t'_x} = \frac{\frac{BK + L}{L}}{\frac{BK' + L'}{L'}}$$

Thus, for illustration, if we assign the values indicated in the following table, the relative length of time required to reach half saturation, that is $t_x : t_x'$ would be in the ratios shown by the fractions.

	$B = B' = 1$		$L = L' = 1$	
	$L = 1$	$L' = 2$	$B = 1$	$B' = 2$
K = 0.01	$\frac{1.01}{1}$	$\frac{2.01}{2}$	$\frac{1.01}{1}$	$\frac{1.02}{2}$
$K' = 10.0$	$\frac{11}{1}$	$\frac{6}{1}$	$\frac{11}{1}$	$\frac{21}{2}$

It is here to be seen that, when the coefficient of solubility (K) is small, respiration (L) has little effect on the rate of absorption; but that when the gas is readily soluble, its rate of absorption varies almost in proportion to the respiration. Contrariwise, the rate of absorption of a very slightly soluble gas is mainly determined by the circulation, while the absorption of a very soluble gas is little influenced by variations in the circulation.

V. Technique for determining concentrations of gas in the body. The concentration of any gas at any time in the venous blood is easily determined by breathing repeatedly the air in a small rubber bag, such as a football or basketball bladder. The air in the bag and that in the lungs thus come to have the same partial pressure of the gas to be determined, as that which exists in the venous blood. The concentration of the gas found by analysis in the air in the bag is then multiplied by the coefficient of distribution, and gives the figure for the concentration of the gas in the venous blood.

The total amount of the gas in the entire body may be estimated approximately by multiplying the body weight in kilos by the figure for the concentration in the venous blood in milligrams per liter.

The concentration of any gas in the arterial blood is much less easy to determine experimentally. The most accurate method involves the withdrawal of a sample of blood by means of a hypodermic syringe inserted into an artery, usually the radial artery at the wrist. The respiratory method generally used, that of Haldane and Priestley, consists in making a sudden deep expiration through a tube; the end to which the lips have been applied being immediately closed. A sample of air is then withdrawn from this end of the tube; and as this is the last air expired, it represents a sample from the alveoli of the lungs. The accuracy of the procedure depends upon the subject having continued to breathe uniformly and naturally. There is a strong tendency, however, for the subject to draw an extra deep inspiration just before making the deep expiration. Another technique is also described in this paper. The concentration of the gas in question in the "alveolar air" or "arterial pulmonary" air, as it is more accurately termed, is multiplied by the distribution coefficient and gives the concentration in the arterial blood.

The concentration of a gas in the arterial blood can also be estimated in other special ways. Thus a small amount of air is injected through a hypodermic needle into the peritoneal cavity of the abdomen, or merely under the skin, and after a sufficient time for diffusion a sample is withdrawn and analyzed. It usually affords the figure sought (13). The same end may be attained by holding air in the mouth with the lips closed and cheeks distended; for a partial pressure of any gas in the blood gradually develops in the air in the mouth by diffusion. The length of time that air must be left in the abdomen, or in the mouth or any body cavity, such as

the stomach or rectum, in order to attain equilibrium with the blood varies widely according to the character of the gas, and other conditions.

In the urine also pressures and concentrations of gases occur practically identical with those in the arterial blood at the moment of the secretion of the fluid. But except by catheterizing the bladder, the urine cannot in most cases be obtained quickly enough after its secretion to be of significance in this respect.

Finally, it is important to recognize that, while the distribution of a gas between the blood and the body as a whole usually occurs at a coefficient of unity, the different tissues differ widely. The capacity of fat to absorb gases, not only such gases as ethyl ether but even such gases as nitrogen, is high; while that of bone is probably low. That of the brain, even for the fat solvent gases, is about the same as that of blood.

The organs become saturated at rates which vary in proportion to their blood supply, and which therefore differ widely. The brain and the kidney have especially large blood flow. The amount of the gas in question in the organ can generally be estimated by analyzing a sample of blood withdrawn from the vein draining the organ, and multiplying this concentration by the weight of the organ.

VI. Ethyl iodide, and its advantages for measuring the circulation. We had drawn up a long list of volatile organic substances, in addition to those already mentioned, having properties more or less suitable to the end we had in view. Ethane was next in order; but as it is not on the market we arranged to have it prepared. As a step in this direction, a liter of ethyl iodide was made for us in the laboratory of organic chemistry here. This substance is a heavy, but quite volatile liquid, very slightly soluble in water: specific gravity 1.944 at 15°, boiling point 72.2°. It was immediately evident that ethyl iodide itself was worthy of test; and the test soon showed that it is an ideal substance for use in the measurement of the circulation.

At first we took it in small amounts by stomach, dissolved in a little alcohol and a large amount of water; and determined its rate of elimination through the lungs. It soon became evident, however, that only a minor part of the total amount of the substance swallowed was thrown off in the breath, whereas in similar experiments with ether we had found that the entire amount ingested was exhaled, except for a small portion in the urine. We therefore carried out the following experiment which is reproduced here essentially as it stands in our laboratory notes. It afforded the first definite indication that with ethyl iodide the complications of a gradual saturation of the body, and an increasing return of the substance in the venous blood, are eliminated by the destruction of the substance in the tissues. Presumably it is decomposed into sodium iodide and ethyl alcohol.

Experiment 1. Inhalation of ethyl iodide.

Subject Y. H. Weight 80 kilos. Sitting, pulse 64. Respiration 9 liters per minute. About 0.75 ml. of ethyl iodide were volatilized in a spirometer containing 200 liters of air and mixed by a fan, electrically driven. Subject inhaled from spirometer through mouthpiece and valves, and exhaled into 4 Douglas bags in succession. Air analyzed by means of iodine pentoxide train. Inspired air, iodine figure 100. Expired air discharged to waste for 3 minutes; then collected in bag I for 3 minutes, iodine figure 54; bag II, 3 minutes, iodine figure 50; bag III, 3 minutes, iodine figure 47; bag IV, 5 minutes, iodine figure 49; average 50. (These data show a virtually constant concentration in the expired air for 18 minutes. *Eureka.*) Assuming a dead space of 33 per cent and a coefficient of distribution of 2.0, the alveolar concentration is 25 and that of the arterial blood 50. As the amount absorbed per minute averaged $50 \times 9 = 450$, the circulation figures to $450 \div 50 = 9.0$ liters per minute and the stroke index is 1.76 ml. per kilo per beat.

Experiment 2. Same subject. Respiration, 8 liters per minute. Inhaled from spirometer for 3 minutes. Inspired concentration 100, expired 50. Then rebreathed for one minute into a 2-liter rubber bag. Analyses of inspired and expired air indicated that 1200 units of ethyl iodide had been absorbed. This figure divided by the body weight would give an indicated concentration in the body and venous blood, if no decomposition occurred, amounting to 15 units of ethyl iodide per kilo, and this would correspond to 7.5 units per liter in the venous pulmonary air. Analysis of the air in the small bag showed, however, only 3 of ethyl iodide. In a second rebreathing bag 2 were found.

Experiment 3. Same subject. Respiration 8 liters per minute. Inhaled from spirometer and exhaled into succession of Douglas bags. Concentration inhaled 43. Concentration exhaled at the end of three minutes 19, and at the end of 9 minutes 21. Total amount absorbed 1440. Then after inhaling fresh air free from iodide for 3 minutes, the subject exhaled for a minute into another Douglas bag. Ethyl iodide figure in this bag was 3. Then rebreathed into a small rubber bag and found a concentration of 0.

Experiment 4. Same subject. Experiment similar to above. After inhaling from the spirometer until 2242 units of ethyl iodide had been absorbed the subject then inspired fresh air but collected the expired air in a succession of Douglas bags for 1, 3 and 5 minutes respectively. Bag I contained 24 units of ethyl iodide, bag II, 12 units; and bag III, none; total 36.

Evidently very little of the ethyl iodide absorbed from the lungs returns to the lungs again in the venous blood.

VII. Distribution of ethyl iodide between air and blood in the lungs. The basic idea in this method of measuring the circulation, that is, the volume of blood flowing through the lungs each minute, is as follows: Suppose that, while breathing air containing a certain amount of ethyl iodide vapor, one milligram of the substance is absorbed from the lungs into the blood each minute. Suppose that it is demonstrated that the blood, when it leaves the lungs, contains 0.2 milligram in each liter. Suppose none, or a negligible amount of the ethyl iodide absorbed, comes back to the lungs in the venous blood. Then the amount absorbed per minute divided by the amount in each liter of arterial blood is the volume of the blood flow, that is, the circulation. On these suppositions the circulation is therefore 5 liters per minute; for $\frac{1}{0.2} = 5$.

It is essential to know the concentration of ethyl iodide in the blood as it leaves the lungs. To obtain this figure the most direct method is to draw blood from an artery and to analyze it for ethyl iodide. We have done this on animals, and it is feasible on patients. But aside from the objection to arterial puncture, this method of getting the figure needed involves either the withdrawal of an inconveniently large volume of blood, or the inhalation of a much higher concentration of ethyl iodide vapor than is otherwise needed.

The same information can be obtained by another and less radical procedure. In the capillaries of the lungs the blood is spread in so large a surface, that for most gases a near approach to equilibrium of tension in the alveolar air and in the blood is almost instantly attained. From its general physical constants, it is therefore probable that in the distribution of ethyl iodide an equilibrium between its vapor phase in the alveolar air, and its dissolved phase in the blood would be reached before the blood left the lungs. This we have confirmed experimentally.

As will be shown in another section, a sample of alveolar air can be obtained even without the coöperation of the subject. The concentration of ethyl iodide in this sample can be determined by analysis. All that is needed in addition is the coefficient of distribution, in other words, the specific solubility of ethyl iodide in blood at body temperature. In brief, as nearly as we can determine it, we find that for practical purposes this coefficient is 2. In other words, if a sample of alveolar air is analyzed and found to contain a certain amount of the substance per liter at room temperature (18° to 25°C.), then the amount of ethyl iodide in each liter of blood leaving the lungs is twice as great.

In general the solubility of ethereal vapors in blood is nearly the same as in water, or only slightly less. This is notably true of ethyl ether. The solubility of ethyl iodide vapor in water seems not to have been determined. Even the solubility of the liquid in water is known only roughly (25). Thus we find that, when 5 or 10 ml. of liquid ethyl iodide are shaken in a liter of water at 20°, about 1.3 ml. of the iodide go into solution, or about 2.5 grams in the liter of water. The vapor tension of ethyl iodide at 20° is 100 mm. (Landolt-Börnstein, Physikalisch-Chemische Tabellen). This is equivalent to a concentration of 0.94 gram per liter of air. The distribution coefficient for ethyl iodide vapor in water at room temperature would thus be indicated as approximately 2.66.

In order to determine more exactly the coefficient of distribution we have used two methods, one physical, the other chemical. Both were applied first to water and afterwards to blood. The blood was drawn directly from the heart of a dog by means of a large hypodermic syringe with a long needle thrust through the chest wall. This is neither painful nor injurious. The blood was rendered non-coagulable by means of sodium fluoride.

Physical method. The apparatus employed for the physical determination went through some modifications in the course of this work, for it was found that any stopcock lubricated with vaseline or other greasy substance was liable to leak when exposed to the vapor of ethyl iodide under pressure. Soap was tried as a lubricant also; it was better, but not very satisfactory. The final form of the apparatus is shown in figure 2. In this arrangement of the parts, the stopcocks, during the time of equilibration and pressure measurement, are protected by a layer of fluid, blood or water on one cock, and mercury on another.

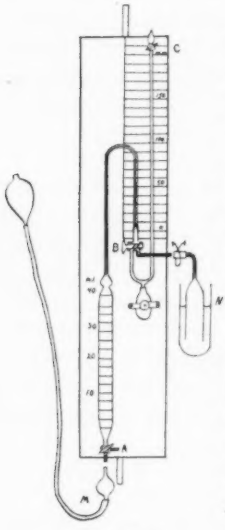

Fig. 2

The apparatus consists of a graduated burette of a total capacity of a little less than 50 ml. connected by a capillary tube to a mercury manometer. There are three stopcocks, *A*, *B* and *C*. Back of the manometer is a mirror graduated in millimeters. The whole apparatus is mounted on a board with an iron rod running along the back of it, so that it can be laid in a rack and rotated in a large water bath at body temperature. Before each period of rotation the mercury is forced up to the top of both limbs of the manometer, and held by closing cocks *B* and *C*. The mercury is raised by squeezing a rubber bulb below the manometer by means of a screw.

For a solubility measurement about 15 ml. of water are drawn into the burette. With all cocks closed the apparatus is rotated for a minute or two on its long axis in the water bath, and is then stood upright in the water at such a height that the entire burette is below the surface of the water, but the scale of the manometer is above the surface. The burette is then vented through the outlet on cock *B*, and again rotated under water in the bath. The initial pressure reading is then made on the manometer with the apparatus vertical in the bath; and the apparatus is then lifted out of the bath and set in a vertical stand on a work bench. Two accessory pieces of apparatus (*M* and *N*), as shown in the figure, are attached at cocks *A* and *B* by means of short pieces of rubber tubing. The accessory piece, *M*, which is connected to the stopcock, *A*, below the burette consists of a small glass bulb, a length of rubber tubing, and a larger counterpoise bulb containing mercury. When the counterpoise bulb is lifted, and the stopcock *A* is opened, mercury can be run into the burette so as to pass the air which it contains out through stopcock *B*. To stopcock *B* meanwhile, has been connected the second accessory piece, *N*, consisting of a small bell tube immersed in a test tube containing liquid ethyl iodide. The air in the main apparatus is passed into this ethyl iodide volatilizer, and drawn back again into the burette. The water or blood is then restored as it was originally by raising the counterpoise mercury bulb and closing the cock, *A*, below the burette. The two accessory pieces are then disconnected, and the whole apparatus is stood in the water bath until it comes to the same temperature as that originally used. The pressure is then read on the manometer.

Usually an increase of pressure of about 100 mm. is found. This represents the

partial pressure of ethyl iodide which has been introduced into the air of the burette. The mercury is then raised to the top of both limbs of the manometer, and for a distance of two or three millimeters into the capillary tube connecting the manometer and burette; and the cocks, *A* and *B*, are turned off. The apparatus is then rotated on its side in the rack in the water bath for 30 to 50 seconds, after which it is stood upright in the bath, and the pressure again read. This process is repeated until no further change of pressure occurs. The drop in pressure indicates the amount of ethyl iodide that has gone into solution under the pressure of ethyl iodide remaining in the air. It tends to accuracy to have the volume of air and liquid in the burette in such relation (about 2:1 for ethyl iodide) that the drop of pressure is about one-half the initial partial pressure of the vapor.

The results of some determinations of the solubility of ethyl iodide in water, at room temperature and near body temperature, are shown in the accompanying table. Using the letters at the heads of the columns, the formula for calculating the solubility, as expressed by the coefficient of distribution, from such data is

$$\frac{d \times b}{a \times c} = K$$

The coefficient of distribution K is the figure by which the concentration of ethyl iodide vapor in the air is multiplied in order to find the concentration in solution. For water at 37°, the relation is:

$$\frac{\text{Concentration in Vapor}}{\text{Concentration in Solution}} = \frac{1}{1.87}$$

Using only the figures which are significant, K for water at 37°C. is therefore 1.9.

This technique was then applied to blood. The only change of procedure from that used on water consisted in filling the burette beforehand with air from a small rubber bag, whose contents had been rebreathed half a minute. This air contained about 5 per cent CO_2, and 15 to 17 per cent oxygen. The blood was fully equilibrated with this atmosphere in the burette, and repeated readings were made on the manometer, before the figure for the initial pressure was accepted as correct. Without this preliminary equilibration with respired air, the blood might take up or give off oxygen or CO_2 during the later equilibration with ethyl iodide vapor. Then the accessory pieces of apparatus (M and N in the figure) were connected, and the determination of the solubility of ethyl iodide vapor was carried out exactly as on water. The results of three determinations are shown in table 2.

These determinations were made early in our investigation and merely as a preliminary to testing out the general availability of ethyl iodide for measuring the circulation. Accordingly, after that work was well developed and the feasibility of the method demonstrated, we returned to

the determination of the coefficient of distribution in blood, for the purpose of getting a more exact constant for routine use. To our surprise, figures as high as 3 or even 4 were obtained in this second series. In fact when the rotation of the apparatus in the water bath was continued for 10 or 15 minutes, instead of a solubility of 2 or a little more, such figures as 6, 7, 8, and in one case 14, resulted.

TABLE 1

Solubility of ethyl iodide vapor in water at various temperatures

TEMPERATURE	VOLUME OF WATER	VOLUME OF AIR AND VAPOR	PARTIAL PRESSURES OF ETHYL IODIDE VAPOR			COEFFICIENT OF DISTRIBUTION (E.I. IN AIR = 1)
			Before equilibration	After equilibration	Difference	
°C.	*a* ml.	*b* ml.	mm. Hg	*c* mm. Hg	*d*	*K*
22.5	14.8	29.0	90.0	39.0	51.0	2.55
23.0	14.0	28.0	80.0	35.0	45.0	2.57
36.0	14.0	30.4	130.0	68.0	62.0	1.98
36.0	16.3	33.2	80.5	39.0	41.5	2.17
37.0	13.0	31.4	128.0	74.0	54.0	1.76
37.0	14.0	28.0	106.0	56.0	50.0	1.78
37.0	14.0	28.0	133.0	65.5	67.5	2.06
Average for 37°.....						1.87

TABLE 2

Solubility of ethyl iodide vapor in blood at body temperature

TEMPERATURE	VOLUME OF BLOOD	VOLUME OF AIR AND VAPOR	PARTIAL PRESSURES OF ETHYL IODIDE VAPOR			COEFFICIENT OF DISTRIBUTION (E.I. IN AIR = 1)
			Before equilibration	After equilibration	Difference	
°C.	*a* ml.	*b* ml.	mm. Hg.	*c* mm. Hg	*d*	*K*
37.0	14.7	29.7	92	44	48	2.20
37.5	18.7	25.7	63	25	38	2.09
38.0	16.6	27.8	134	53	81	2.56

It thus became evident that, in addition to the solution of ethyl iodide, a progressive decomposition of the amount already dissolved was occurring. Presumably this reaction is a hydrolysis resulting in ethyl alcohol and hydriodic acid, and is followed immediately by the neutralization of the acid by alkali drawn in small part from sodium bicarbonate, but chiefly and ultimately from the main alkali reserve of the blood, the alkali protein of the corpuscles. The process is indicated by the equation

$$C_2H_5I + H_2O + (NaHCO_3 + \text{alkali protein}) = C_2H_5OH + NaI, \text{ etc.}$$

If ethyl alcohol is thus produced, practically all of it must remain in solution; for its amount is minute and its tension in the vapor above the blood would be only a small fraction of a millimeter. Apparently the same decomposition occurs when ethyl iodide is merely dissolved in water, although in this case the rate of reaction is very much slower. The speed of reaction both in water and in blood is extremely variable and seems to be affected to a high degree by the extent to which the fluid under equilibration runs over the surface of glass. The catalyzing action of glass surfaces on ethyl iodide in solution even in water appeared particularly in the results by the other method described below.

The reaction by which ethyl iodide is decomposed in blood and in water seems not to be an oxidation; nor to be considerably opposed by its products; nor to be greatly affected by such slight changes of pH as may occur in normal blood. In support of the first of these statements an experiment may be instanced, in which blood was saturated with carbon monoxide, and the burette of the apparatus was filled with that gas. After equilibration in the water bath at body temperature, the vapor of ethyl iodide was drawn in, and the distribution of the substance was determined as usual. The resulting figure was 1.7 after an equilibration of 30 seconds, but rose to 5 when the equilibration was continued for several minutes. On the second point above mentioned an experiment was tried in which three or four drops of ethyl alcohol and a small amount of sodium iodide were added to the blood. The blood was then equilibrated with rebreathed air and ethyl iodide vapor as usual. A distribution of 3 was obtained. On the third point mentioned, experiments were carried out in which CO_2 was first bubbled through the blood, and the air in the burette was enriched with this gas. Figures approximating 6 for the distribution of ethyl iodide between vapor and solution under such conditions were obtained, after the apparatus was rotated for several minutes in the water bath.

Chemical method. Under these circumstances it seemed essential to verify, and if possible render more exact, the coefficient of distribution for blood by some other method. The general technique employed involved determinations on much lower concentrations than those in the physical method, and analysis of both vapor and solution by means of the iodine pentoxide train.

Glass sampling tubes were used of the type shown in figures 4 and 6, capacity 0.5 liter, 35 cm. long and 4.5 cm. in diameter, with small tubes at the end. The ends were closed by bits of rubber tubing clamped short. Glass stopcocks cannot be used as the grease upon them absorbs ethyl iodide vapor.

Three small drops of liquid ethyl iodide were put into such a tube, and volatilized by placing it in the water bath. Then 5 ml. of water were added. The tube was vented to relieve pressure, and then rotated in the water bath at temperatures between 37° and 38°C. for various lengths of time as noted below. It was next held vertically in the air while the needle of a glass hypodermic syringe was thrust through

the rubber tubing on the lower end and most of the water was drawn off. By the same technique, but with a larger syringe, 11 or 12 ml. of air were drawn off from the upper end. When used with care, an all glass hypodermic syringe may be used in this way to handle gases or liquids as accurately as a pipette or burette. It has the advantage that its contents can be introduced into a closed system by thrusting the needle through the side of a rubber tube; for the hole closes again tight against moderate pressure.

Both the air and the water samples obtained in this way were then analyzed by means of the iodine pentoxide apparatus. The air was shot in by sticking the needle of the syringe through a rubber tube leading from outside the building to the iodine pentoxide U-tube. The water was similarly introduced into a bulb, where 5 or 6 liters of air were drawn through it, causing it to bubble vigorously, and to be in part evaporated in the course of four minutes. This stream of air then passed through the iodine pentoxide. The iodine liberated from the pentoxide was caught in potassium iodide solution and titrated with sodium thiosulphate solution, as usual. The iodine figures given in table 3 are expressed in units of 0.1 ml. of thio.

TABLE 3

Relations of iodine figures for equal volumes of water and air after equilibration in a rolling tube containing 500 ml. of air and 5 ml. water at 37° for various lengths of time

TUBE ROTATED	IODINE FIGURES		RELATION (E.I. IN AIR = 1)
	Water	Air	
20 seconds	88	42	2.10
2 minutes	116	54	2.15
5 minutes	177	48	3.7
5 minutes	135	40	3.4
15 minutes	55	8	6.9

The results by the chemical method afford approximately the same coefficient of distribution in water at 37°C. as that found by the physical method. They indicate also, however, the readiness with which, under some conditions, ethyl iodide may decompose. It is otherwise a comparatively stable substance of its class. The results of the chemical method when applied to water were as shown in table 3.

When this method was applied to blood, the decomposition was so rapid that, after rolling the tube for only 30 seconds, the iodine figure from the analysis of the blood was four times as large as that from the air. When the tube was rotated ten minutes, the figure was ten times as large. It is to be noted that alcohol is removed from solution in water or blood only with difficulty, even by a very large current of air; and the blood in these tests was only evaporated about half way to dryness. Calculation shows that alcohol on passage through iodine pentoxide produces only two-thirds as much iodine as does ethyl iodide; in other words, the iodine figures for the two substances stand in the relation of 2:3; this is largely because of the iodine which the latter substance itself contributes. That alcohol is

formed in the decomposition of ethyl iodide is indicated by an experiment in which a solution stood at room temperature for two days. The iodine figures at the beginning and end of this period, obtained by drawing air through samples of the solution and then through iodine pentoxide, were respectively 22 and 14, or about 3 to 2. With these facts in mind we modified the equilibration as follows:

After three small drops of liquid ethyl iodide had been volatilized in the equilibration tube in the water bath at a temperauure of 38°C., a sample of about 12 ml. of the air and vapor was drawn into a Luer syringe; and the tube was vented momentarily by opening one of the pinch cocks on one of its ends, so as to bring its contents and those of the syringe to atmospheric pressure at the temperature of the bath. A cork was stuck on the end of the needle of the syringe to close it for the few minutes before 10 ml. of its contents were analyzed. Meanwhile 5 ml. of water or blood were introduced into the equilibration tube by means of another Luer syringe; and the fluid was allowed to run down the length of the tube, while it was held at a slope of about 30 degrees in the bath. The other end of the tube was then raised so that the blood ran back over the same line, no new glass being wetted. This process was repeated a certain number of times, each tilting of the tube taking about 3 seconds. Then the tube was lifted out of the bath and held vertically, while the greater part of the water or blood was drawn into the syringe. From the syringe 3 ml. were injected into the bulb in front of the iodine pentoxide apparatus, and the iodine figure was determined. Care was taken to make sure that all volatile organic substances (ethyl iodide, alcohol, etc., see section on analysis) were ventilated out of the blood, and drawn through the tube of iodine pentoxide. A blank experiment showed that the original blood gave an iodine figure of zero, indicating no content of volatile organic substance. By varying the number of tiltings in otherwise identical experiments the results shown in table 4 were obtained.

From the data of table 4 it seems quite certain that the coefficient for blood at body temperature in vitro is not more than 2.1, and that it may be slightly less. It is to be noted that, if alveolar air is measured and analyzed after it has come to room temperature, it will have contracted so that 200 ml. will contain about the same amount of ethyl iodide as 210 ml. of this air at the temperature of the lungs. We therefore decided to use the figure 2 in all work on the circulation; but the use of the figure 2 in practice virtually recognizes 2.1 as the true coefficient at body temperature. Similarly, if 2 were shown to be the true figure controlling the distribution of ethyl iodide between air and blood at body temperature in the lungs, then 1.9 would be about the right figure to use in deriving the blood concentration, when the alveolar air is analyzed at room temperature. If 1.9 were used, the circulation would be calculated as about 5 per cent greater than when the coefficient of solubility 2 is applied. As the ethyl iodide method of measuring the circulation is scarcely accurate to better than 5 per cent, the use of 2 is justifiable.

Equilibration in vivo. For a final check and confirmation of these measurements, the experiments on dogs, described in section X, were

utilized. Simultaneous determinations were made by the iodine pentoxide method on samples of alveolar air, arterial blood, and venous blood drawn from the right heart. Control analyses of the blood were made before the inhalation of ethyl iodide, and showed no perceptible amount of any volatile organic substance, such as acetone, reacting with iodine pentoxide. In two experiments 0.1 liter of air was injected initially into the peritoneal

TABLE 4

Iodine figures for water and blood, when the fluid was equilibrated with air containing ethyl iodide vapor at 38° by merely tilting the tube in a water bath, the number of times here shown

NUMBER OF TILTINGS	IODINE FIGURES IN 0.1 ML. OF THIO			RELATION (E.I. IN AIR = 1)
	Water	Blood	Air	
2	20		10.5	1.90
3	40		21.0	1.90
6	29		14.0	2.09
8	27		13.0	2.08
3		93.0	50.0	1.86
6		120.0	58.0	2.07
8		30.5	14.5	2.10
11		53.0	25.0	2.12
20		23.0	8.0	2.90

TABLE 5

Relations of iodine figures from equal volumes of alveolar air, arterial blood, venous blood and abdominal air. Data obtained from dogs inhaling ethyl iodide vapor

ALVEOLAR AIR a	ARTERIAL BLOOD b	VENOUS BLOOD	ABDOMINAL AIR	RELATION $\frac{b}{a} = K$
35.0	74.0	13.0		2.11
22.5	42.0	3.0	0	1.87
9.0	19.0	2.0		2.11
13.5	27.5	3.0	0	2.09
14.5	28.0			1.93
14.0	27.0	3.0		1.93
14.0	30.0			2.14
Average				2.02

cavity; and a sample of this abdominal air was withdrawn after the inhalation of ethyl iodide, and analyzed. The data obtained by these procedures are shown in table 5.

Not a trace of ethyl iodide was found in the abdominal air. This fact is a striking demonstration of the destruction of the substance in the body. In previous papers we have shown that the concentration of CO_2 in ab-

dominal air rises to that corresponding to the alveolar air and arterial blood, and that ether vapor, which is not decomposed at all in the body, reaches the same level (13).

For the venous blood the iodine figures were usually about 10 per cent of those for the arterial blood. But it seems likely, from the agreement of the coefficient of distribution in vivo and in vitro, that the volatile substance which came off from the blood in these cases was in part, or largely, alcohol into which the ethyl iodide had been decomposed, after diffusing from the blood into the tissues. If a part of this alcohol escaped back into the blood, and thus came to the lungs, its enormous coefficient of solubility (about 1500) would prevent the escape of any appreciable amount into the alveolar air.

The average relation of the iodine figures for the alveolar air to those for the arterial blood is 1:2.02. Perhaps a small part of the ethyl iodide had already split up into alcohol and sodium iodide, during the two or three minutes that necessarily elapsed between the withdrawal of the blood from the carotid artery and its injection into the analyzer train. It appears however that the decomposition in blood, when not treated as in our equilibration experiments, is quite slow. If some hydrolysis does occur it would make the distribution coefficient appear a little smaller than it really is; for the iodine figure for a molecule of alcohol is only two-thirds of that for a molecule of ethyl iodide. On the other hand, the iodine figures for the venous blood suggest that the coefficients calculated from the alveolar air and arterial blood are really too large by about 10 per cent. If the coefficient used in calculating the circulation were made smaller, the estimate of the circulation would work out correspondingly larger. The comparison of results by the ethyl iodide method with those by the Fick method in section X indicates, however, that the use of the coefficient 2.0 (corresponding to 2.1 at body temperature) is not too large.

In general the agreement of the relation found in vivo with that found in vitro (tables 4 and 5) is quite satisfactory. It demonstrates that no decomposition occurs in the lungs sufficient to invalidate the equilibration of alveolar air and pulmonary blood on a coefficient of distribution lying very close to 2.

VIII. Method of analyzing for ethyl iodide in air and blood. The method employed for the determination of small amounts of ethyl iodide in air, and in blood and other fluids, is essentially the same as that developed in this laboratory (14) for the estimation of ethyl ether by oxidation with iodine pentoxide. The use of iodine pentoxide for the analysis of carbon monoxide is well established. We find that it is equally applicable and convenient for many of the volatile hydrocarbons.

The air sample containing ethyl iodide vapor is drawn through a U-tube filled with glass wool and iodine pentoxide powder, and heated in an oil

bath. The iodine pentoxide oxidizes the ethyl iodide, and the iodine liberated from both substances is collected in potassium iodide solution. The iodine is then determined by titration with sodium thiosulphate, with starch paste as the indicator. One molecule of ethyl iodide is oxidized with the liberation of 3.6 atoms of iodine. The reaction agrees with the equation:

$$10\ C_2H_5I + 13\ I_2O_5 = 20\ CO_2 + 25\ H_2O + 36\ I$$

Apparatus. The apparatus used for the estimation of ethyl iodide by this method is shown in figure 3. By means of suction a stream of air is drawn at the rate of 1.0 to 1.5 liters per minute through the entire analyzer train. The air is admitted

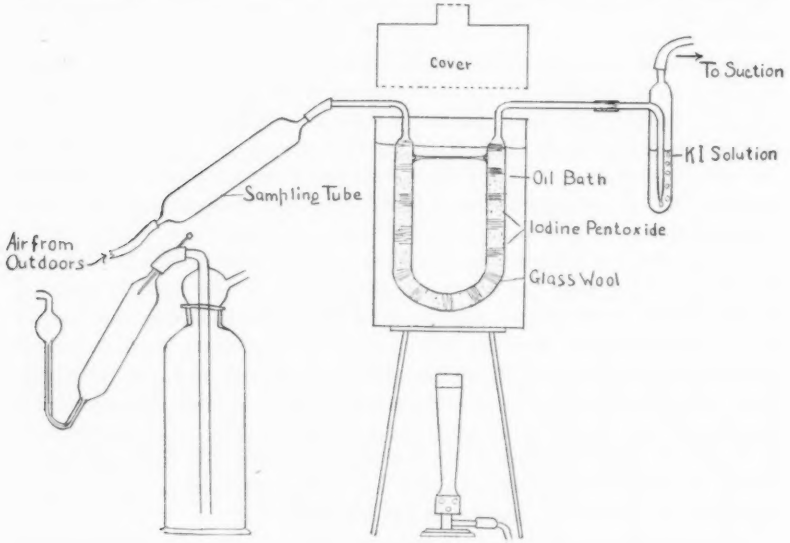

Fig. 3

through a tube at the left of the apparatus, which terminates outdoors in an area where the atmosphere is free from smoke or automobile exhaust gas; otherwise an automobile passing in the street may cause error, for the carbon monoxide which it produces is burned by iodine pentoxide with a liberation of iodine. Air from the room must not be drawn into the apparatus. The flame of a Bunsen burner impinging on a metal surface produces sufficient carbon monoxide in the air round about to invalidate the analytical results.

The U-tube is filled with alternate layers of glass wool and iodine pentoxide to a total of about 40 to 50 grams of the latter. The tube is made of pyrex glass, 25 cm. in height and 20 mm. in diameter. The side arms are of 5 mm. tubing and extend 12 cm. laterally. The tip of the tube on the exit side is tapered to make an interlocking glass joint with the absorption tube, which is connected to it by a short piece of rubber tubing. The oil bath, in which the U-tube is immersed, is made of sheet copper electrically welded at the seams; it measures 30 cm. in height, 15 in width, and

10 in thickness. "Crisco" makes a satisfactory oil for the bath. A small sheet metal ventilating hood containing a suction fan is placed over the bath to remove the fumes from the hot oil.

If the U-tube breaks in the hot oil bath a reaction of sufficient violence occurs between the iodine pentoxide and hot oil to blow the contents of the bath into the face of the analyst. As this accident might cause severe burns or even loss of eyesight, there should be a copper cover to direct the hot oil away from the analyst.

Conditioning the pentoxide. It is necessary to condition the iodine pentoxide, before it can be used for analytical purposes. This is done by keeping the temperature of the oil bath at 250°C. for several hours, while a stream of air is drawn through the pentoxide. A considerable amount of iodine is usually driven off by this heating. Different specimens of pentoxide, even of the best quality, require different lengths of time for conditioning; but with a good preparation 12 to 24 hours should be sufficient. When the conditioning is complete, the potassium iodide solution in the absorption tube should show no more than a barely detectable yellow tinge, when pure air is drawn through the pentoxide at 200°C. for 30 minutes. Even the best preparations of iodine pentoxide decompose to some extent spontaneously, so that it may be necessary to deduct the amount found in a blank run. If the conditioning has been thorough, this blank is small and remains constant from day to day.

We strongly advise the use of iodine pentoxide prepared by the chloric acid method (15). Its spontaneous decomposition is far less than that of the substance made in other ways, in fact it is doubtful whether any other can be used satisfactorily for this purpose.

Method of analysis. The oil bath is maintained at a temperature ranging from 160° to 200°C. While the bath is heating, a stream of air is drawn through the train to remove any iodine, which may have separated in the pentoxide. When the temperature has reached 160°, the absorption bulb is washed out with 5 per cent potassium iodide solution, and charged with 8 to 10 ml. of the solution. It is then connected with the U-tube of pentoxide and with the suction tube. The current of air is run for 5 minutes. At the end of this time the absorber is removed and the iodine solution carefully washed into an Ehrlenmeyer flask of 300 ml. capacity. The liberated iodine is then titrated with a solution of sodium thiosulphate of the strength defined below, with starch paste as indicator. Unless the conditioning of the iodine pentoxide has been very thorough, it is advisable to run two consecutive blank determinations; these should agree within 0.05 ml. of thiosulphate and neither should exceed a total of 0.15 ml. Close agreement is usually found in blank determinations; but if this is not the case, it is necessary to repeat the procedure, until consistent results are obtained.

The stream of air is drawn through the iodine pentoxide continuously, except for the brief interruptions while inserting and removing the absorption tube. If the stream is discontinued for any length of time, the small amount of iodine which separates spontaneously, when iodine pentoxide is hot, accumulates in the U-tube; and this iodine is carried into the absorber, when the current of air is started again, thus invalidating the accuracy of the analysis. Whenever the absorber is removed, even for a few seconds, the rubber tube connected to the source of the suction is slipped over the tapered end of the U-tube.

In the analysis of air containing the vapor of ethyl iodide, the sampling tube holding it is inserted in the train at the point shown in figure 3. In most of the work described in this paper the sampling tubes used were of 0.25 liter capacity and closed at each end with short pieces of rubber tubing and pinch clamps. Sampling tubes fitted with glass cocks cannot be used, for in a few minutes the grease with which these cocks are lubricated removes appreciable amounts of ethyl iodide from the air

within. After the sample tube is inserted and the pinch clamps are removed a stream of air is drawn through the sampler and washes its contents through the U-tube of iodine pentoxide. A run of 5 minutes with a flow of air of at least 1 liter a minute is sufficient to carry all of the ethyl iodide into the pentoxide, and to flush all of the liberated iodine into the absorber.

So large a current of air involves no escape of iodine through even the simple absorber used. The analyst may assure himself of this fact by inserting a second absorber in the train. The current must be rapid.

When the substance under examination is a liquid, the apparatus shown standing below the sampling tube in figure 3 is inserted in the train at the point usually occupied by the sampling tube. This apparatus consists of a glass tube of 20 mm. bore and 10 cm. length, with a short length of 5 mm. tubing on one end and a capillary of 1 mm. bore, 10 cm. long on the other. The capillary is bent to an acute angle with the larger tube and has a small bulb near its extremity. Beyond this bulb is a short piece of 5 mm. tubing. The tube from the bulb is connected to the fresh air inlet; the tube from the opposite end is connected by a short rubber tube to a gas washing bottle of 0.25 liter. capacity. The side tube from this bottle is connected to the U-tube of iodine pentoxide and the air stream from out doors is drawn through this apparatus. The fluid under examination is measured in an accurately calibrated hypodermic syringe, and its needle is inserted obliquely through the rubber connection joining the two portions of the apparatus. The fluid is then delivered into the body of the tube and the needle is withdrawn. The stream of air passing through the blood carries it in a series of films which travel up the tube and break at the connection of the gas washing bottle; a very thorough aeration of the fluid is thus obtained. Any fluid which is carried beyond the tube is trapped in the wash bottle. It is very important that blood shall not be drawn into the pentoxide tube; if this accident occurs the process of conditioning, as described above, must be repeated.

A run of five minutes usually serves to remove all of the ethyl iodide from 5 ml. of blood or other fluid aerated in the manner here described. It is advisable, however, to remove the absorber at the end of this time, to insert another charged with potassium iodide solution, and to continue the aeration of the fluid for an additional five minutes. If the aeration is complete the potassium iodide solution in the second run shows, when titrated, no more free iodine than the normal blank. If there is more than this amount, it should be added to that found on the first run; and additional runs of five minutes should be made, until no more ethyl iodide comes off from the blood, and the liberation of iodine from the pentoxide drops to its own spontaneous rate of decomposition.

In analyzing fluids the absorber must be disconnected from the U-tube before the suction is shut off; otherwise the slight suction caused by the fluid in the sample tube may draw the potassium iodide solution from the absorber back into the iodine pentoxide tube; a serious mishap.

When the sample of fluid or air under analysis contains more than 3 or 4 mgm. of ethyl iodide, the liberated iodine may separate as a crystalline deposit in the arm of the U-tube connected to the absorption tube. *This deposit must be volatilized near the close of the analysis by passing the flame of a Bunsen burner along the tube, so as to heat it slightly and thus to transfer this iodine to the absorber.* The largest amount of ethyl iodide which the apparatus will handle conveniently in a single analysis is 5 to 6 mgm.; larger amounts cause such a deposit of iodine crystals that it is difficult to remove them completely. When the run is finished the absorption bulb is removed; and the solution is washed into a flask, and titrated with a solution of sodium thiosulphate.

The thiosulphate solution used is very dilute; a convenient strength is one in which each milliliter is equivalent to 0.573 mgm. of iodine and hence to 0.2 mgm. of ethyl iodide. The thiosulphate solution may be standardized to this strength against a freshly prepared solution of iodine. But this is not necessary, for in using this method of analysis for the determination of the rate of the circulation, the exact strength of the thiosulphate is of no importance, so long as the same solution is used in the titration of all of the air and blood samples taken from a single experiment. The amount of thiosulphate used to titrate the iodine liberated from each sample is applied, without conversion into the corresponding amount of ethyl iodide, to calculate the rate of the circulation. Unnecessary calculation is thus avoided. A thio solution corresponding approximately to that suggested for the blank determination is made by dissolving 1.1 grams of crystalline sodium thiosulphate in 1 liter of water. The unit thereafter is 0.1 ml. of this solution. The absolute value of this unit may be ignored.

IX. Calculation of results. The use of the purely relative unit, suggested in the preceding paragraph, makes the arithmetical calculation of the circulation of the blood by this method extremely simple. Thus, for example, if all the samples of air analyzed in one experiment are of the same volume (we generally use 0.25 liter), and if the titration figures for the inspired, expired, and alveolar samples are respectively, 10.0, 5.0 and 2.5 ml. of thio, then taking 0.1 ml. as the unit,

$$\frac{100-50}{25} \times \frac{\text{respiration}}{2} = \text{circulation}$$

Here respiration, known from the spirometer readings, and circulation are measured in liters per minute, while 2 in the denominator is the coefficient of distribution of ethyl iodide between blood and air in the lungs.

Here also we may point out the simplest method, as we think, of calculating the oxygen consumption and CO_2 output from such an arrangement of apparatus as that employed with the ethyl iodide method. The inspired air is measured by means of the spirometer readings for five or ten minutes, and is divided by the time, so as to afford the number of liters per minute. This observed volume is then reduced to the volume which it would have at 0° and 760 mm. Ordinarily at sea level during normal weather and at the common American laboratory temperature, about 20°C., it is sufficient merely to multiply the observed volume by the factor 0.91.

A sample of expired air is drawn from the mixing bottle and analyzed for CO_2 and oxygen. Doubtless in most laboratories and clinics a mercurial apparatus, either the original Haldane or Henderson's modification, will be employed; but it is our practice to use the Henderson-Orsat (16) which with care is quite accurate enough and much less liable to accidents. Analysts should fix their attention more upon attaining reliability than upon extreme accuracy. We have seen analysts who strain their eyes to read the gas burette to the last hundredth of a milliliter, but who neglect to take sufficient care to assure completeness in the absorptions. Reliable gas analysis, as applied to expired air, has two requisites: first, that the apparatus should

be tested frequently by analyzing pure atmospheric air, and by deriving and applying the necessary correction, if the result for the sum of the oxygen and CO_2 differs appreciably from 20.96 per cent; and second, that every reading after an absorption should be checked by passing the gas again into the absorbent.

Owing to the fact that as a rule less, but occasionally more, CO_2 may be exhaled than would correspond to the volume of oxygen absorbed during a respiration experiment, the expired air usually has a volume somewhat different from that of the inspired. For this reason the analytical figures cannot be applied directly to those for the volume of respiration, but have to be modified by means of a factor which derives the amount of oxygen that was inspired from the nitrogen that is expired. The oxygen found in the expired air is then subtracted from this virtual inspired oxygen. The difference is then divided, not by the volume of the sample of expired air, but by the calculated volume that the sample must have had when it was inspired. This division gives the percentage of oxygen absorbed from the inspired air.

There are other ways of making this correction than that here shown, but they involve the use of several factors, instead of the single fixed factor for the relation of oxygen to nitrogen in pure atmospheric air. The following is an example of this method of calculation and registration of results:

Method of calculating oxygen consumption, output of CO_2, and respiratory quotient when only the inspired air is measured and the expired air is analyzed

(*a*) Respiration, or volume of air inspired per minute at prevailing barometer and temperature, and saturated with water vapor =	7.550	liters
(*b*) Corrected volume of inspired air, $a \times \frac{\text{bar.} - \text{water vapor}}{760} \times \frac{273}{273 + \text{temperature}}$, or (at 760 mm. and 20°), $a \times 0.91$ =	6.87	liters
(*c*) Volume of sample of expired air for analysis =	24.72	ml.
(*d*) Volume after absorption of CO_2 =	24.01	ml.
(*e*) Volume of residual nitrogen after absorption of oxygen =	19.66	ml.
(*f*) Volume of CO_2 in sample, or c-d =	0.71	ml.
(*g*) Volume of oxygen in sample, or d-e =	4.35	ml.
(*h*) Oxygen in a volume of pure air corresponding to the amount of nitrogen shown in (*e*), or $e \times \frac{20.93 \text{ (oxygen in pure air)}}{79.04 \text{ (nitrogen in pure air)}} = e \times 0.2648$ =	5.20	ml.
(*i*) Virtual volume of air sample before it was inspired, or $e + h + 0.03$. (Here 0.03 is the CO_2 in pure atmospheric air) =	24.89	ml.
(*j*) Percentage of CO_2 produced, or $\left(\frac{f \times 100}{i}\right) - 0.03$ =	2.82	per cent
(*k*) Percentage of oxygen consumed, or $\frac{h - g}{i} \times 100$ =	3.415	per cent
(*l*) Output of CO_2 per minute, or $\frac{b \times j}{100}$ =	0.194	liter
(*m*) Consumption of oxygen per minute or $\frac{b \times k}{100}$ =	0.235	liter
(*n*) Respiratory quotient or $\frac{l}{m}$ =	0.826	

X. Accuracy of ethyl iodide method tested against Fick method. In order to determine whether or not the ethyl iodide measurement of the circulation is reliable and accurate it is desirable to check it against some absolute standard. The nearest approach to such an absolute standard, that is available, is that offered by the Fick method in experiments on animals.

For this purpose dogs were used. In most cases morphine and urethane, both non-volatile hypnotics, were administered together with local anesthesia by cocaine. The carotid artery was exposed for the withdrawal of samples of arterial blood. Venous samples were taken directly from the right heart by means of an all glass syringe and a long needle thrust through the body wall. A cannula tied into the trachea was connected with double valves like those used in our work on man, but smaller. The inspiratory valve was connected to a spirometer, and the expiratory valve led to a mixing bottle. Alveolar air was obtained as described in section XI. In fact the ethyl iodide method was applied in all respects as we use it on man, except that in these experiments the concentration of the vapor administered was 10 times as high. This is still far below the anesthetic concentration of ethyl iodide. The higher concentration is necessary in order that the amount of the substance in a 10 ml. sample of blood shall be accurately determinable by means of iodine pentoxide.

The oxygen and CO_2 in the expired air were determined by means of the Henderson-Orsat gas analyzer. The oxygen and CO_2 in the blood samples were determined with the Van Slyke apparatus. The inspired, expired and alveolar airs were analyzed for ethyl iodide by means of the iodine pentoxide train.

Owing to the number of determinations of three different types to be made, it was not possible to keep the animals in sufficiently good condition, so as to afford a measure of the volume of the circulation during health. In fact with the exception of experiments 1, 5 and 6, vitality was distinctly decreased, and the animals were in a more or less shocked condition. Even in these cases the arterio-venous oxygen and CO_2 differences (not included in the table, but readily obtainable from its data) are probably much larger than normal, and indicate that the circulation was correspondingly slower than in dogs during normal life. This condition is, however, of no importance in the present connection, except as illustrating the nature of circulatory depression. The point of the experiments is the comparison, which they afford, of the values obtained for the circulation, when calculated from the blood gases and from ethyl iodide. The results of seven complete experiments are given in table 6.

In this table, after the numbers of the experiments in the first column, the second column shows the respiration, that is, the volume of air breathed per minute at room temperature, varying from 2.0 to 17.0 liters per minute.

This air was inhaled from a large spirometer containing air in which 0.025 ml. of liquid ethyl iodide had been volatilized per liter of air. The pulse rates in the third column run from 48 to 216 per minute. The conditions of the animals in respect to respiration and circulation were therefore quite widely varied, and afford correspondingly various tests of the agreement of the two methods.

In the fourth and fifth columns are given, in liters per minute at 0°C. and 760 mm., the amount of CO_2 exhaled and the amount of oxygen absorbed. These figures were obtained by analyzing samples of expired air for oxygen and CO_2, and multiplying the percentages by the volume of air inhaled, with corrections for temperature and pressure, and for the decrease in the volume of the air due to the respiratory quotient.

TABLE 6

Comparison of measurements of the circulation by means of ethyl iodide and by Fick method. Data obtained from same dogs as in table 5

EXPERIMENT NUMBER	RESPIRATION PER MINUTE	PULSE PER MINUTE	CO_2 OUTPUT PER MINUTE	O_2 CONSUMPTION PER MINUTE	BLOOD GASES				IODINE FIGURES			CIRCULATION PER MINUTE		
					Arterial		Venous					Fick method		Ethyl iodide method
					CO_2	O_2	CO_2	O_2	Inspired air	Expired air	Alveolar air	CO_2	O_2	
	liters		*liter*	*liter*	*vols. per cent*							*liters*		
1	3.5	48	0.0546	0.0771	35.0	22.2	38.6	17.5	95.0	51.5	41.0	1.52	1.64	1.86
2	17.0	140	0.249	0.302	40.0	17.0	47.0	8.5	80.0	57.0	52.0	3.56	3.54	3.79
3	2.42	150	0.0652	0.0805	44.5	17.0	51.5	9.0	35.0	26.0	9.5	0.931	1.01	1.13
4	2.5	130	0.0671	0.0815	46.0	22.0	55.5	9.5	80.0	60.0	40.0	0.707	0.652	0.625
5	2.0	80	0.061	0.0919	41.0	14.0	45.0	9.5	70.0	33.0	20.0	1.525	2.08	1.86
6	4.0	132	0.065	0.093	36.0	24.0	40.5	19.0	27.0	17.0	11.0	1.445	1.86	1.80
7	3.7	216	0.0885	0.121	48.5	22.0	55.0	14.0	32.0	21.5	14.0	1.36	1.51	1.39
Averages												1.58	1.76	1.78

In the sixth to ninth columns are the volumes per cent of CO_2 and oxygen found by analysis in the arterial and venous blood. The data in columns four to nine are all that are needed for the Fick method. The arterial CO_2 is subtracted from the venous CO_2 and the difference is divided into the CO_2 output (in column 4). The quotient is the circulation, expressed in liters of blood per minute, passing through the lungs. Similarly the venous oxygen is subtracted from the arterial oxygen, and the difference is divided into the oxygen consumption (column 5); and the quotient in this case also indicates the circulation in liters per minute.

In columns ten, eleven and twelve are given the relative concentrations of ethyl iodide in the inspired, expired and alveolar air. The expired concentration is substracted from the inspired, and the difference is divided by

the alveolar concentration. This quotient is then multiplied by one-half the volume of air breathed (from column 2), and gives the circulation in liters per minute. The reason for dividing the respiration by 2 in this calculation is that the coefficient of distribution for ethyl iodide between the air and blood in the lungs is 2.

The results by both methods are given in the last three columns to the right, and are averaged below. The agreement of the averages for the circulation calculated from ethyl iodide with that from oxygen is remarkably close. The figure for the circulation calculated from CO_2 is not so closely in agreement. The discrepancy even here is not very large, considering the number of factors involved in a Fick determination; it probably arises from slight inaccuracies in the determination of CO_2 in the blood.

The general results of these experiments definitely confirm the reliability of the ethyl iodide method. They also validate the use of 2.0 as the coefficient of distribution for use in calculating the concentration of ethyl iodide in the blood from that found in the alveolar air sample.

XI. A METHOD FOR OBTAINING ALVEOLAR AIR AND SOME OF ITS APPLICATIONS. The method of measuring the circulation here described is rendered much easier, and more exact than it would otherwise be, by a simple device by which 0.25 liter of alveolar air is obtainable in the course of 5 or 10 minutes. It requires no voluntary assistance by the subject. This device or technique has, however, a wider possible use than merely as a part of the ethyl iodide method, and merits a few words of comment and suggestion.

In 1905 Haldane and Priestley (17) discovered that alveolar air may be obtained by a deep expiration through a long tube. They demonstrated that the last portion of the air in the tube comes from deep in the lungs, and that the partial pressure of CO_2 in this air is a physiological constant. That work has probably had a greater effect, and today underlies a greater extent of important knowledge, than any other one paper in the field of physiology in modern times. It is the cornerstone of our present conception of respiration and the hydrogen ion concentration of the blood, and of the regulation of these functions. Yet the significance of the alveolar air is probably not even now generally or fully realized. If it were, it would not be thought necessary, except on a much smaller number of cases than at present, to draw a blood sample from a patient's vein, and to analyze this blood for its content of CO_2 as an index of blood alkali. Instead, we should merely obtain the alveolar air; and we should determine the partial pressure of CO_2 in this air expressed in millimeters. That figure multiplied by 1.25 gives the number of volumes per cent of CO_2 in the blood, and thus the alkali bicarbonate in use in the blood, with a degree of accuracy which is quite sufficient for all ordinary purposes. This statement is based on the fact that, except in extremely acute disease, the hydrogen-

ion concentration of the blood varies so little from a normal figure, that it may be treated as practically a constant (18). As this constant is the resultant of the pressure of CO_2 and of the blood alkali, the amount of alkali is calculable by merely multiplying the pressure of CO_2 by a known factor, namely, 1.25.

The reason that this method of determining the blood alkali is not generally used is probably that the technique of Haldane and Priestley requires the voluntary assistance of the subject. That to be here described merely requires that the subject breathe as naturally as possible through a mouthpiece. At first the breathing under such conditions tends to be slightly abnormal, usually in the direction of overbreathing; but in the course of 3 to 5 minutes, normal respiration returns, and nearly normal alveolar air is usually obtained. To compensate the slight overbreathing the factor can be increased to 1.3. This technique is conveniently combined with determinations of the respiratory exchange and basal metabolism, as well as with measurement of the circulation with ethyl iodide.

The apparatus used is shown in figure 4. It consists of a mouthpiece with inspiratory and expiratory valves; they are of the same general type as are used in determining the respiratory exchange and indirect calorimetry. But in addition there are two small outlet tubes. One of these outlets is just beyond the expiratory valve, the other opens from the chamber between the inspiratory and expiratory valves. To these outlets are connected two small rubber tubes, one leading to a gas sampling tube, and the other to a Müller valve below the sampling tube. Thus they form a circuit so arranged that a small part of the air just beyond the expiratory valve is drawn back, during each inspiration, into the sampling tube by the slight negative pressure in the chamber between the inspiratory and expiratory valves.

This device is novel only as regards its automaticity and the use of inspiration as its motive force. Otherwise it is identical in principle with the technique used by Krogh and Lindhard (21) and other investigators (22) in which air is taken at the end of normal expiration. This procedure has been criticized as less accurate than that of a single deep expiration. But this objection applies less in regard to ethyl iodide, for the purpose here in view than to the determination of the normal alveolar CO_2. The latter cannot be obtained except during normal breathing; while in respect to ethyl iodide it is sufficient to determine the concentration actually in the lungs, even if respiration at the moment is not quite normal.

The metal and rubber parts of such valves as we use are shown in figures 4 and 5 The shell and covers can be made by any tinsmith out of thin sheet copper, which is afterward tinned. They form a cylindrical can 2½ inches in diameter and 2 inches high. A partition divides the can into two equal chambers. To an opening on the side of one of these chambers is soldered a flattened tube ½ inch high, 1¼ inches wide

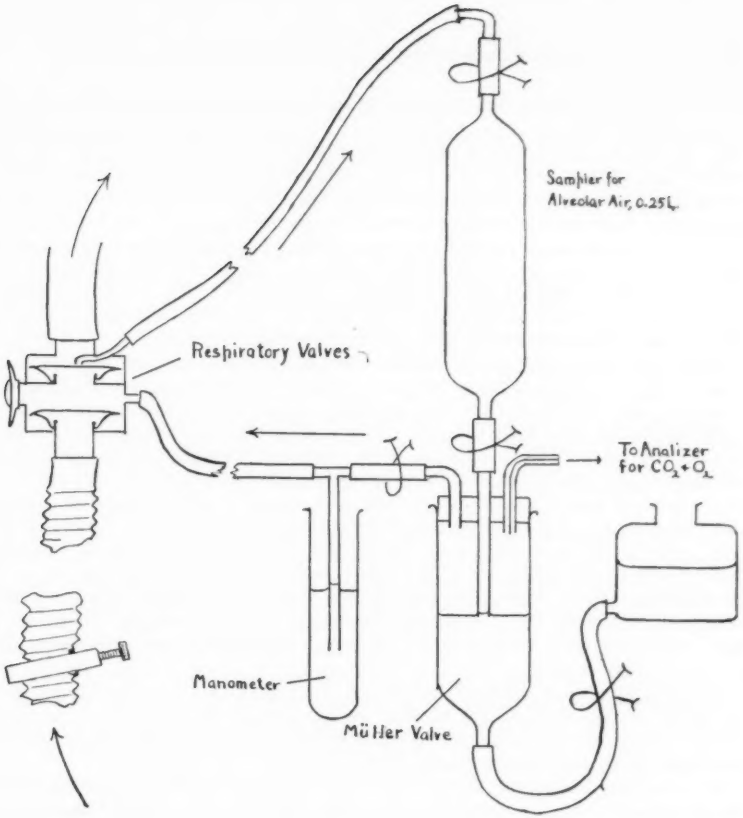
Sampler for
Alveolar Air, 0.25L.
Respiratory Valves
To Analizer
for $CO_2 + O_2$
Manometer
Müller Valve

Fig. 4

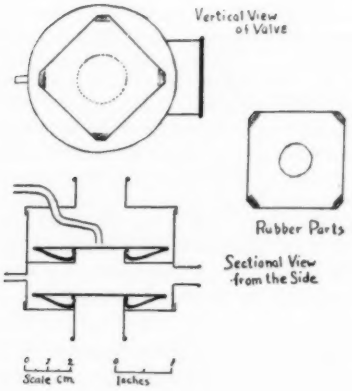
Vertical View
of Valve
Rubber Parts
Sectional View
from the Side
Scale cm.
Inches

Fig. 5

and 1 inch long. In the middle of the partition is a round opening $\frac{3}{4}$ inch in diameter, on one side of which a piece of tube $\frac{3}{4}$ inch in diameter and $\frac{3}{16}$ inch long is soldered. The tube is flared a little at the free end, or a wire is soldered around it, to hold the rubber valve to be described below. The covers for each end of the can have similar round openings $\frac{3}{4}$ inch in diameter, to which are soldered similar pieces of tubing projecting 1 inch outside the cover. To these are later attached the large rubber tubes, through which the subject of the experiment breathes. The tube on one of these covers projects also $\frac{3}{16}$ inch to the inside, and is flared exactly like that in the central partition. Through the other cover a small copper tube $\frac{1}{8}$ inch bore is passed so as to project 1 inch outside, while inside the cover it is bent as shown, so that its end is $\frac{1}{16}$ inch above the middle of the rubber expiratory valve.

The two rubber valves are made of sheet rubber of about three times the thickness of dental dam, or $\frac{1}{32}$ inch. Each of the two valves is made of two pieces of this rubber 2 inches square. In the middle of one of each of these two pieces a round hole $\frac{1}{2}$ inch in diameter is cut; thus these holes are a little smaller than the tubes over which they are to be stretched. The four corners of the perforated piece are fastened to those of the other square piece with no hole with rubber cement and pressed with a warm iron. The hole in the lower piece of one of these valves is stretched over the flange of the tube in the middle partition, so as to hold the upper piece against the end of the metal tube to form the expiratory valve. The other pair of rubbers is similarly placed on the flanged end of the tube on the inside of one of the covers and serves as the inspiratory valve. The adjustment is shown in figure 5. Such valves are easily made; they offer very little resistance even during heavy breathing, and are quite tight. The covers are made airtight on the can by wrapping a strip of adhesive tape around the edge of each.

Corrugated, noncollapsible rubber tubing of 1 inch bore is best for the lower or inspiratory connection of the valves. In the arrangement for the circulation method, air is drawn through it from a spirometer, in which a minute amount of ethyl iodide has been volatilized in a large volume of air.

To the upper or expiratory side of the valve a piece of smooth bore, $\frac{3}{4}$ inch rubber tubing 3 or 4 feet long is fastened. If alveolar air is to be obtained, corrugated tubing cannot be safely used at this point, because of the cross currents which the corrugations set up. This expiratory tube may go to a spirometer, or to a Douglas bag, or to a mixing bottle; the latter arrangement is used in the circulation method.

To the small copper tubes or nipples on the breathing valve are attached pieces of rubber tubing of $\frac{3}{16}$ inch bore, 3 feet long. That from the upper or expiratory chamber leads to the upper end of a glass sampling tube of 0.25 liter capacity held vertically. From the lower end of the sampling tube another rubber tube leads to the Müller valve. When it is desired merely to obtain alveolar air, and the circulation is not to be measured, this sampling tube is omitted and the rubber tube is connected directly to the Müller valve. A counterpoise bulb containing water permits adjusting the level of the water in the valve, so that the end of the inlet tube just touches the surface of the water. The valve operates therefore under a minimum of pressure in one direction during inspiration, but allows no back flow during expiration. The counterpoise bulb is also used as the means for passing some of the air in the Müller valve over into a gas analyzer for the determination of its content of CO_2 in the alveolar air. To the outlet of the Müller valve are connected a manometer and the rubber tube from the nipple on the middle chamber of the respiratory valves.

With this arrangement even the slight resistance of the inspiratory valve is generally sufficient to cause a small volume of air (25 to 50 ml.) to be

drawn through the Müller valve at each inspiration. If this is not the case, the corrugated inspiratory tube is narrowed by means of a screw clamp. During expiration the Müller valve having practically the same pressure at each end does not allow the passage of any air. During inspiration the last portion of the previous expiration remains in the upper chamber of the respiratory valve and in the large smooth bore rubber tubing. Accordingly, the current of air drawn through the Müller valve during inspiration takes from the expiratory tube a small sample of the last air expired during the previous expiration. The sampling tube is thus gradually filled with air from the end of each expiration. This air comes from the deeper portion of the lungs; and unless respiration is abnormally shallow, it is therefore alveolar air. In normal persons its content of CO_2 is usually found to lie between 5.0 and 5.8 per cent, which is the concentration, as is now well established, corresponding to a normal blood alkali in healthy persons at sea level.

The sampling tube above the Müller valves has a capacity of 0.25 liter, and it is generally necessary for a resting subject to breathe through the apparatus for 5 to 10 minutes in order to flush this tube thoroughly, so that it contains undiluted the alveolar concentration of ethyl iodide vapor. The determination of this concentration as exactly as possible is essential to accurate measurement of the circulation.

To insure correctness on this point, it is our practice to analyze for CO_2 a sample of air taken from the Müller valve at the end of each observation. If the content of CO_2 found is approximately the normal alveolar percentage of the individual, this fact assures us that the sampling tube has been flushed sufficiently, and contains the true alveolar concentration of ethyl iodide vapor. A sample of the individual's normal alveolar CO_2 is quickly obtained by removing the sampling tube and leaving only the Müller valve in circuit with the respiratory valve.

XII. The respiratory dead space and its relation to alveolar gas concentrations. In this field it is very important to obtain checks on such quantities as those of the gases in alveolar air by some collateral measurements. The best check on the calculated alveolar concentration of any gas is that obtained from analysis of a sample of arterial blood. Bock and Field (19) have supplied this check for the alveolar CO_2 when obtained by the Haldane and Priestley method. We have applied it to the concentration of ethyl iodide in the alveolar air and in arterial blood of dogs (see section X), and have found agreement on the basis of a coefficient of solubility of 2.

Another check or set of checks, which gives information of value for its own sake also, is that obtained from calculation of the respiratory dead space. Let us call D, the dead space, expressed as a decimal fraction (i.e., 0.3, 0.4) of the volume of the breath; I, the concentration of any gas

in the inspired air, expressed in per cent of the dry gases; E, the similar concentration in the expired air; and A, that in the dry alveolar air. Then for CO_2 the relations of the dead space to the alveolar concentration are:

$$D = \frac{A - E}{A} \tag{1}$$

$$A = \frac{E}{1 - D} \tag{2}$$

That the dead space can be most conveniently and quite accurately determined by means of ether vapor, using such respiratory apparatus as in our present investigation, has been shown by us in a recent paper (20). Calculation of the dead space by means of ether does not involve determination of an alveolar value as a datum. When a low concentration of ether is contained in the inspired air, and this atmosphere is breathed for only two or three minutes, practically all of the ether which reaches the lungs is absorbed, and the concentration is therefore virtually zero. The ether in the expired air comes almost wholly from that which has merely entered and been again blown out of the dead space. It thus affords an index of the volume of the dead space in relation to the volume of the breath. It affords an excellent check on the alveolar value used in any other method of calculating the dead space, as for instance those from CO_2 and from ethyl iodide. The formula for obtaining the dead space by means of ether is

$$D = \frac{E}{I} \tag{3}$$

With a gas or vapor of comparatively low solubility such as ethyl iodide, the relation of the dead space to the inspired, expired and alveolar concentrations of that vapor becomes

$$D = \frac{E - A}{I - A} \tag{4}$$

and the alveolar value is

$$A = \frac{E - (I \times D)}{1 - D} \tag{5}$$

We have used these relations extensively in convincing ourselves that the concentration of ethyl iodide in the air of the lungs becomes fully equilibrated with the blood as it passes through the pulmonary vessels, and that this air is truly represented by alveolar samples obtained as above described. Much hangs on this point, although it is not absolutely essential. Before the technique for obtaining relatively large volumes (0.25

liter) of alveolar air was developed, it was our practice to calculate the alveolar concentration of ethyl iodide by an indirect method, as follows: The dead space was first estimated from CO_2, or better from ether, by means of equation 1 or 3, and this value was then applied in equation 5. This calculation yields a figure for the concentration of ethyl iodide in the alveolar air which can be used, together with the inspired and expired concentrations, for calculating the volume of the circulation exactly as described in section IX. The average of a series of determinations thus made was about the same as that of a series in which the alveolar concentrations of ethyl iodide were obtained directly from analysis of the air in the sampling tube and the Müller valve; but the variations in the individual determinations, when the alveolar concentrations were calculated indirectly, were much greater than when the alveolar air was obtained and analyzed.

The general concordance of the results of the direct and indirect determinations indicates that the alveolar values experimentally obtained are reliable and sufficiently exact. But even now, if in a measurement of the circulation doubt of its correctness arises, it is our practice to calculate the dead space from the alveolar and expired CO_2 and also from the inspired, expired and alveolar concentrations of ethyl iodide. If the results are identical or differ by not more than a few per cent the doubt is resolved. If, on the contrary, there is a large discrepancy that measurement of the circulation is discarded.

XIII. Ethyl iodide method for measuring the circulation in man. The measurement of the circulation in man by means of ethyl iodide requires that we determine four quantities: *1*, the volume of air inspired per minute; *2*, the concentration of ethyl iodide in this air; *3*, the concentration of the substance in the expired air; and *4*, the concentration in the alveolar air. From the first three data we obtain the amount of the substance absorbed per minute. This quantity is then divided by the concentration in the arterial blood, which is twice that found in the alveolar air.

The apparatus employed is shown in figure 6. It includes a spirometer of a capacity of at least 100 liters, that which we use contains 400 liters and is graduated so that is can be read accurately to 0.7 liter, or 1 per cent of the respiration for a ten-minute period; it is one of those made by the American Meter Company, New York City, for use in the laboratories of city gas companies for the standardizing of their gas meters. It must be painted with red lead both inside and out; for ethyl iodide reacts with exposed metals. A rotary fan must be installed and must be kept running inside of the spirometer throughout a determination; this fan must be driven by an electric motor outside the spirometer.

The method for making the extremely dilute vapor to be inhaled is as follows: A rubber stopper with one large hole is put into the inlet tube of the spirometer. Into this hole in the stopper is inserted loosely, so that it can be turned easily, the small tube on the end of one of the 0.25 liter gas sampling tubes, or the end of a 100

ml. pipette with the tip cut off. For each 100 liters of air about 0.3 ml. of liquid ethyl iodide is pipetted into the sampling tube or large pipette, which is held horizontally. Then while an assistant pulls down on the counterpoise weight of the spirometer, the sampling tube or large pipette is rotated so as to moisten as large an area as possible of the interior surface of the tube or pipette bulb with liquid ethyl iodide. Under these conditions the air, which is drawn through the sampling tube or large pipette, soon carries all of the ethyl iodide into the spirometer in vapor form. Additional air is then drawn in until the spirometer is full, and the fan is started.

At the outlet of the spirometer is a large three-way cock so that the tube which leads to the person under test, and through which he inspires, can either draw air from the room or from the spirometer. Just beyond this cock a small side tube permits the withdrawal of samples of the air and ethyl iodide vapor that the subject inspires from the spirometer. The sampling tube has a capacity of 0.25 liter; it is

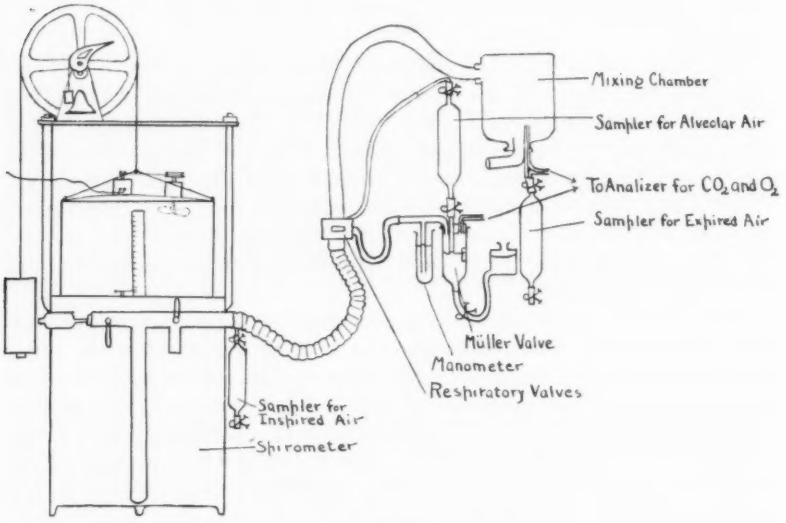

Fig. 6

filled with water initially which is drained off to draw in the air sample. From the three-way cock a corrugated noncollapsible rubber tube about a meter in length leads to the respiratory valves and mouthpiece. To these valves is also attached the sampling device for alveolar air described in section XI. From the respiratory valves a rubber tube of 2 cm. smooth bore leads to the side tabulation of a five liter aspirator bottle supported upside down. This bottle serves as a mixing chamber for the expired air. From it the sample of expired air is drawn in a 0.25 liter sampling tube for analysis of its content of ethyl iodide. (For method of analysis, see section VIII.) A sample is also taken from the mixing bottle for analysis of its content of oxygen and CO_2 as data for the calculation of the respiratory metabolism and indirect calorimetry. (For method of calculation, see section IX.) A stop watch is a considerable convenience in this work.

The course of an experiment is as follows: The subject is placed under the desired conditions. He may be lying flat or head down, sitting or standing, in a tub of hot water, on a stationary bicycle and riding easily, or with a heavy load on the Prony brake. His pulse is counted every few minutes until its rate is constant, and at the beginning, middle and end of the period of observation. During rest it is felt at the wrist; during work on the bicycle ergometer, the observer's finger is placed on the carotid artery, just under the angle of the jaw; or a stethophone may be strapped to the chest and wires led from it to a telephone receiver in another room.

A clip is put on the subject's nose, and the mouthpiece of the respiratory valves is put between his lips and teeth. During this time the three-way cock on the spirometer is turned to admit fresh air for him to inspire. The scale on the spirometer is read. The Müller valve on the alveolar air device is examined to make sure that it bubbles vigorously during each inspiration; and if necessary the corrugated inspiratory tube is narrowed by a screw clamp; but the pressures on the water manometer should never exceed plus and minus 3 cm.; usually 2 cm. are sufficient. Then the stop watch is started, or the time otherwise noted, and the three-way cock is turned so that the subject inspires from the spirometer.

After the subject has breathed through the system for some minutes a sample of expired air is drawn from the mixing bottle and its contents of CO_2 and oxygen are determined. Toward the end of the ten minute period of inhalation from the spirometer, samples of the inspired and expired air are drawn in the sampling tubes for analysis for their content of ethyl iodide. Half a minute before the end of the period the alveolar sampling tube is also shut off by means of spring clips. The duration of a test should be ten minutes during rest; but five minutes is sufficient during work and deep breathing.

At the end of the period (best read on a stop watch) the three-way cock is turned to fresh air, and the spirometer is read. The mouth piece is removed from the subject's lips, and the clip from his nose. A sample of air is drawn from the Müller valve and analyzed for CO_2, to insure that the sampling tube has been thoroughly flushed and filled with alveolar air. The three samples of air, inspiratory, expiratory and alveolar in the 0.25 liter tubes, are analyzed for ethyl iodide. The results are calculated in the way described in section IX. A complete observation including the filling of the spirometer, the period of test, analysis of the expired air for oxygen and CO_2 (during the inhalation) and the analyses of the three samples of air for ethyl iodide can be carried out by one experienced operator inside of half an hour. It is better, however, to have an assistant, and for one person to analyze for CO_2 and oxygen and the other for ethyl iodide. Under these conditions not only the entire experiment, but all the calculations can be performed in half an hour.

The amount of iodine absorbed by the subject during a test is somewhat less than 150 mgm. or its equivalent 180 mgm. of sodium iodide, a quite insignificant amount pharmacologically.

In the course of the development of this method, and while seeking the most convenient arrangement of the apparatus, we have accumulated a number of observations extending over several months on one man under the ordinary conditions of working about the laboratory during the morn-

TABLE 7

Showing extent of variation of observations on a single subject under approximately uniform conditions at different times

Subject Y. H. Weight 80 kilos. While working about the laboratory he sat down in a chair for five minutes before each observation

DATE 1924–25	PULSE PER MINUTE	RESPIRATION PER MINUTE	CIRCULATION PER MINUTE	STROKE VOLUME
		liters	*liters*	*liter*
November 26 (a)	70	7.0	8.8	0.126
November 26 (b)	75	8.8	10.0	0.133
November 29 (a)	64	7.0	9.4	0.147
November 29 (b)	62	6.6	7.6	0.122
December 2 (a)	68	8.5	9.3	0.137
December 2 (b)	68	8.0	8.8	0.129
December 30	62	8.25	8.15	0.132
January 20	63	6.4	8.97	0.139
January 22	64	8.0	8.5	0.133
January 27 (a)	60	8.0	7.9	0.131
January 27 (b)	65	10.4	8.6	0.132
February 11	60	9.2	8.33	0.139
Average				0.133
Maximum				0.147
Minimum				0.122

ing. For these tests he merely sat down in a chair for a few minutes. These observations are reported in table 7. They show both the degree of uniformity of behavior of the circulation under approximately the same conditions at different times, and also the extent of experimental variation of results under such conditions obtained with the ethyl iodide method. None of the measurements here reported are appreciably more than 10 per cent above or below the average figure of 0.133 liter for the stroke

volume of the ventricle of the heart of this man under these conditions. The method is, therefore, accurate to within this range of error on a single determination and somewhat more closely when duplicate determinations are performed; preferably on separate days.

XIV. Significance of data of the circulation. The circulation has been heretofore so nearly entirely unmeasurable that few well defined terms, and no system, are available in which to present data. Without such terms and system the significance of data is not easily seen. In the closely related field of the respiratory exchange and indirect calorimetry the central conception is that of basal metabolism: the volume of oxygen consumed and the corresponding number of calories produced per square meter of surface in a certain time, at rest in the post-nutritive state. The circulation, respiration, production of heat, and performance of work are all merely parts of the one general process of the body's energetics.

Any really convenient system, in which to present and consider data of the circulation, must therefore bring out its relations to the other parts of this general process. Thus the *circulatory index* under basal conditions would be the volume of blood flowing in one minute through the lungs of a man, woman or child in the post-nutritive state and recumbent, per square meter of the body's surface. From such data as we have now the normal value of this index may be estimated as a first approximation at about 3.0 to 4.0 liters per minute.

The minute is a more convenient unit of time than is the hour in dealing with the circulation, although the hour is generally used for indirect calorimetry. For normal subjects under basal conditions the average heat production is about 40 calories per hour per square meter, or 0.666 calory per minute. This amount of heat is produced by the consumption of about 0.139 liter of oxygen. When 3.5 liters of blood transport 0.139 liter of oxygen from the lungs to the tissues the arterial blood must contain about 4.0 volumes per cent more oxygen than the venous. This *arterio-venous oxygen difference*, or for a shorter term the *oxygen difference*, is therefore a quantity which expresses the relation of the activity of the circulation to the intensity of the calorific and respiratory processes. During quite vigorous, but not extremely strenuous, muscular exertion we find that the oxygen difference rises to about 8 volumes per cent. If we take the oxygen capacity of the blood as 20 volumes per cent, the so-called *percentage utilization* of this capacity under basal conditions is thus 17.5 and during muscular work 40 or more. If the oxygen capacity is taken at the usual standard, 18.5 volumes per cent, the basal percentage utilization is 18.9 and that of work 43.2 or more. The oxygen difference expressed in volumes per cent, seems to us a simpler conception for ordinary use than the percentage oxygen utilization, for the latter depends upon the amount of hemoglobin in the blood of the particular subject.

The *stroke volume* of the heart (to adopt the expressive German term) is the fraction of a liter of blood that each ventricle discharges in one beat. It is calculated by dividing the pulse into the circulation; but it is not very enlightening to learn that two individuals have stroke volumes of 0.09 and 0.13 liter respectively, unless their weights and pulse rates are also taken into account. Therefore, in order to express the relative amplitude of the strokes of the heart in different individuals and in the same individual at different times, we have used the *stroke index* as a convenient conception. It is obtained by multiplying the body weight in kilos by the pulse rate in beats per minute and dividing the product into the circulation. In other words, it expresses the stroke volume of each ventricle of the heart at the time of observation in milliliters of blood per kilo body weight. As already stated, we find that for normal persons the stroke index has a value during rest of 1.3 to 1.8, but it may rise to 3.4, or perhaps even more, during vigorous muscular work.

We would also suggest that the time has come to recognize that the respiration and the circulation of a man are quantitative functions and to use these terms in the sense of definite volumes, measured in liters per minute, of air and blood respectively. The number of times a minute that a man breathes is of little significance by itself; nor is the amplitude or volume of each breath by itself of much value. It is the volume per minute, the product of amplitude and rate, that expresses the amount of respiration with quantitative significance. Thus a statement to the effect that "the *respiration* is 7.5 liters per minute" expresses a datum of real value and direct usefulness.

In the same way the term *circulation* should, we think, be used hereafter in a quantitative sense and should replace such locutions as "minute volume of blood flow." With the ethyl iodide method it becomes possible, as it has not been heretofore, to determine, for instance, that the circulation of an athlete at rest is 7.5 liters, and during a race or game it is 30 liters per minute; or again to show that a cardiac patient has a circulation of 5 liters per minute during rest, and only 15 liters during an exertion that taxes him to the limit, with a correspondingly decreased power, in comparison to the athlete, to transport oxygen.

Along with the volumetric data contained in the following tables it would have been easy to combine measurements of arterial pressure. The product of a volume per minute and a pressure gives the work in kilogram meters performed by the heart. But in this initial paper it has seemed advisable not to include the relatively well understood element of blood pressure, but to confine the description, discussion, and conclusions to the volumetric aspects of the heart's behavior. It may be estimated roughly, however, from the data here reported and from general information regarding arterial pressure, that the maximum power of the heart is

about equal to the amount of work involved in lifting the weight of the body one meter per minute.

In collecting the data of the circulation here reported we have not adhered to one strictly defined condition, such as the basal state, but have sought rather to make a general survey of the variations in the circulation during the ordinary activities of ordinary life. Along with the data of each individual, it has seemed advisable, however, to give an estimate of the normal oxygen consumption under basal conditions by the DuBois standard for his height and weight.

XV. Extent of variations in the circulation. The persons whom we have examined and whose data are contained in tables 8 to 12 inclusive fall into four groups: 1, University athletes, 3 members of the crew and 2 members of the soccer team. 2, Men of 30 to 52, 6 members of the faculties of this and other universities, all active laboratory workers. 3, Five women ranging from a girl of 17 to a woman of 50, all healthy and vigorous. 4, Ambulant cardiac patients, 2 women and a man; the man partially digitalized, but working regularly at his trade.

Members of the first two groups were generally observed in the recumbent and standing positions, and also while performing such an amount of work on a bicycle ergometer as could be maintained for a considerable time. The gravitational effect upon the circulation in some cases is very striking, as shown particularly in H. T. K. and B. M. S. in table 8 and A. V. B. in table 9. The first two are athletes, while the third man takes no regular exercise and his muscles are not much developed. In such cases it is seen that the stroke of the heart may be twice as large in the recumbent as in the standing position. In other cases the stroke is only 25 or 30 per cent greater in the one position than in the other; but the effect is evident to some degree in nearly all subjects. This gravitational effect upon the stroke volume of the heart was first observed by Doctors Bock and Field who called it to our attention. We therefore leave the discussion of this phenomenon to be dealt with by them.

To make the adjustment necessary when muscular work is performed, and particularly to meet the increased demand for oxygen, the circulation has three reserves: *1*, increase of pulse; *2*, increase of the stroke volume; *3*, increase of the arterio-venous oxygen difference, or percentage utilization. The data here indicate that these reserves are drawn on in this general order. For an exertion no greater than the ordinary activities of life about one's home and office, or a walk at an easy pace, increase of the pulse is the chief compensation; while the amplitude of the stroke of the heart and the amount of the oxygen difference are only slightly affected. As soon, however, as such moderate activity is exceeded, increase of stroke volume and of oxygen difference are also involved, along with a further increase of the heart rate. The increase of stroke seems to reach its limit—

but on this point further evidence is needed— at a stroke index of about 3.5 ml. per kilo, an oxygen consumption of about 2 liters, and a circulation of about 35 liters per minute. Beyond that point the oxygen difference in the blood increases, because the circulation does not. The strain on the subject then increases to the subject's limit of power. The demand is supplied from the third reserve, but this involves a decrease of the partial pressure of oxygen in the tissues, and an increasing oxygen deficit. Such an oxygen deficit has to be made good after the termination of the period of work (24).

In one experiment it was found that an hour after the vigorous muscular exercise involved in playing tennis for an hour and a half, the circulation was decreased to the lowest figure at any time observed in that subject (Y. H., table 9).

A comparison of the effects of external heat, in the forms of a hot air bath and a hot water bath, with those of work was carried out on one subject (tables 9 and 10). The circulation is seen to increase in both conditions, but in the hot tub the effect was chiefly to increase the pulse, while the stroke volume was not so much affected. This observation is in accord with the fact that, during muscular work with its increase of oxygen consumption and CO_2 production, venous pressure is greatly raised; on the other hand, no such rise of venous pressure occurs from the effects of heat. Contrary to the conditions during exercise, heat increases the circulation much more than the oxygen consumption; the arterio-venous oxygen difference falls therefore very low. Thus a Turkish bath affords a thorough oxygenation of the tissues.

An incidental observation was made on the effect of extra-systoles on the circulation. Owing to an over-indulgence in coffee a few days before the experiment, the subject was at the time losing about 12 beats out of 92, the rate while he was in hot water (table 10). Yet the circulation was maintained at the same volume that it would have had at 92 normal beats per minute. This observation indicates that during the occurrence of extra-systoles the succeeding larger beats of the heart make good the deficit in the circulation for the dropped beats.

The data for the women observed are contained in table 11. They were all examined in a sitting position and usually three or four hours after a light breakfast. Their circulations, in proportion to body weight and height, are essentially like those of the men in the second group.

The most striking feature of the observations on the three cardiac cases reported in table 12 is that, even while they were sitting still, their circulations and stroke volumes were so much smaller than those of normal persons that the arterio-venous oxygen differences were distinctly higher than normal. In this we see an explanation of why such patients are "out of breath" from a degree of physical exertion which in normal persons involves

no considerable decrease of the partial pressure of oxygen in the tissues. Even under resting conditions they are drawing on the third reserve.

It does not seem advisable to complicate the presentation of so much new material by attempting a discussion of points on which there may be differences of opinion. But it may be pointed out that it will be necessary to correlate the variations of the stroke volume of the heart with two other measurements and observations: 1, with measurements of venous pressure, and more particularly with the effective diastolic filling pressure, that is, the difference between the venous and intrapleural pressures; and 2, also with x-ray observations. The evidence from the shadow of the heart indicates that the diastolic volume of the ventricles is not increased during muscular work (23). If then the stroke volume is increased, the gain must be made by a more complete contraction and a smaller residual volume of blood in the ventricles at the end of systole.

Such matters of cardiac mechanics should be much easier of profitable attack now that normal man, and all the variety of experimental conditions which disease offers, may be made the subject of observation in place of the drugged and operated animals or "fragments of animals" from which we have had to extrapolate our conceptions of the circulation heretofore.

XVI. SUMMARY AND CONCLUSIONS

Ever since Harvey showed that the blood circulates, the determination of the volume of flow per minute has remained the outstanding, unsolved problem of the circulation. It is not merely general knowledge that is needed, but rather the means of determining the functional efficiency of the circulation in individuals in all conditions; in other words, a simple and fairly accurate method for measuring the circulation in man. The method based on absorption of nitrous oxide from the lungs is inaccurate and of limited applicability. The Fick method, as modified for application to man, is too elaborate and requires too much in the way of respiratory gymnastics by the subject for use except in research.

Investigations in this laboratory have led to the formulation of the principles controlling the absorption of gases by mere solution from the lung air into the blood. These principles show that the rate of absorption of a very soluble gas, such as ether or alcohol vapor, is dependent mainly on the volume of air breathed, that is, the respiration; while the rate of absorption of a relatively slightly soluble gas is more nearly proportional to the volume of the blood stream through the lungs, that is, the circulation. Gases of about the proper solubility were therefore tried, one after another, until the vapor of ethyl iodide was tested. With this substance a simple, accurate and reliable method for measuring the circulation in man has been developed. It is applicable to practically all conditions.

The advantages of ethyl iodide are as follows: 1, Minute amounts of this substance vaporized in air can be determined accurately by the iodine pentoxide method; and very low concentrations of the vapor may therefore be used. 2, The distribution coefficient of the vapor between air and blood at body temperature is a conveniently low value, approximately 2.0. 3, The substance is decomposed so nearly completely in the tissues of the body, that the amount in the venous blood returning to the lungs may be taken as virtually zero. This property eliminates one of the principal difficulties of methods of this type. 4, No coöperation on the part of the subject is necessary, other than to breathe as naturally as possible through a mouthpiece and valves for ten minutes.

The factors determined are: 1, the volume of air breathed per minute; 2, the content of ethyl iodide in the inspired air; 3, the content in the expired air; and 4, the concentration in the alveolar air. The first factor is multiplied by the difference between the second and third, and gives a, the amount of ethyl iodide absorbed per minute. The fourth factor is multiplied by the coefficient of solubility, and gives b, the amount in the arterial blood. Then a divided by b gives the volume of the blood flow through the lungs, in other words, the circulation.

A procedure for obtaining 0.25 liter of alveolar air has been developed. In addition to its use with this method it has other applications.

A complete determination of the respiratory exchange, with the oxygen consumption as a basis for indirect calorimetry, is made with each measurement of the circulation. The whole process, the period of inhalation, and the analyses for oxygen, CO_2, and ethyl iodide can be completed by one operator in half an hour.

Results have been obtained on a variety of persons, ranging from athletes to cardiac patients. For purposes of comparison and estimation of efficiency, the most significant features of the data reported are the stroke index, or volume in milliliters of blood per kilo body weight discharged by the heart at a beat, and the arterio-venous oxygen difference. The stroke index for normal persons during sitting rest ranges from 1.3 to 1.8. In some individuals it is much larger in the recumbent position—up to 2.0 or more; and much smaller in the standing position—down to 1.0 or less; these observations confirm those of Bock and Field. During vigorous exercise the stroke index may rise to about 3.5.

The oxygen difference in normal persons during rest is about 4 volumes per cent. This means that the circulation is normally so large in relation to the oxygen consumption of the body that the venous blood returning to the right heart contains on the average only 4 volumes per cent less oxygen than the arterial blood. The blood stream pumped by the heart is thus about twice as large as was formerly supposed. During bodily rest its volume in liters per minute is about equal to the volume of air breathed

per minute. During exercise respiration may increase 8 or 10 fold, and the circulation 4 or 5 fold.

Hot baths quicken the circulation without greatly increasing the oxygen consumption. Thus they further reduce the oxygen difference and raise the pressure of oxygen in the tissues. On the other hand during muscular exertion, which is vigorous but such as can be sustained for a considerable time, involving an oxygen consumption of about 2 liters per minute, the circulation increases from a resting value of 7 or 8 liters per minute for a man of 70 or 80 kilos, up to a flow of 30 liters or more per minute. Under such conditions the oxygen difference also increases, reaching 8 volumes per cent, or 40 per cent utilization, or more. During greater exertion the oxygen difference increases, because the circulation does not.

In cardiac patients the inefficiency of the circulation shows itself in the increase of the arterio-venous oxygen difference above the normal value. The capacity of the circulation to keep the oxygen difference down, and thus to keep the pressure of oxygen in the tissues up, appears to be the limiting factor in the maximum power that can be sustained by athletes at one end of the scale, and by cardiac patients at the other.

Acknowledgments. The expenses of these investigations were defrayed from a fund generously granted by the Board of Control of the Yale Athletic Association, acting through the University Department of Health and its director Dr. J. C. Greenway. Part of the apparatus used was purchased from grants in 1922 and 1923 by the Committee on Scientific Research of the American Medical Association. An essential part of a stethophone was loaned for this investigation by the Western Electric Company.

Several of our colleagues in the Sterling Chemical Laboratory assisted us with valuable advice regarding organic substances for test and their preparation. We are indebted to Prof. A. B. Lamb of Harvard University and to Mr. J. A. Almquist of the United States Fixed Nitrogen Research Laboratory at Washington for a suppl y of the special iodine pentoxide. Dr. H. M. Marvin of the Medical Department of the New Haven Hospital arranged for the observations here reported on cardiac patients. Mr. Malcolm C. Henderson, a Senior in Yale College, prepared the ethyl iodide which we have used and gave valuable assistance in the mathematical presentation of the principles of gas absorption.

To all who have thus assisted us and to those persons who have served as subjects in our experiments, we take this opportunity to express our sincere appreciation.

TABLE 8

Data from 5 University athletes (24) showing variations in circulation and related functions, during rest and work

SUBJECTS, CHARACTERISTICS AND CONDITIONS	PULSE PER MINUTE	RESPIRATION PER MINUTE	OXYGEN CONSUMPTION PER MINUTE	R.Q.	CIRCULATION PER MINUTE	CIRCULATION INDEX PER MINUTE	STROKE VOLUME	STROKE INDEX PER KILO PER BEAT	ARTERIO-VENOUS OXYGEN DIFFERENCE
		liters	*liters*		*liters*	*liters*	*liter*	*ml.*	*vols. per cent*
A. N. W. Captain Yale Crew, 21 years, 185 cm., 84 kilos, 2 hours after breakfast			(basal 0.289)						
After lying down 10 minutes	58	11.0	0.466	0.84	9.08	4.32	0.151	1.86	5.14
Standing at ease	62	12.2	0.404	0.81	6.83	3.25	0.110	1.31	5.92
Riding bicycle ergometer; work, 650 kgm. meters per minute	120	43.0	2.000	0.96	26.20	12.50	0.218	2.60	7.63
H. T. K. Number 6 on Yale Crew, 20 years, 187 cm., 87 kilos; 30 minutes after lunch			(basal 0.295)						
After lying down 10 minutes	50	10.2	0.397	0.99	14.30	6.81	0.286	3.36	2.77
Standing at ease	68	13.2	0.597	0.86	9.79	4.66	0.144	1.65	6.10
Riding bicycle ergometer; work, 1500 kgm. meters per minute	152	84.0	4.600	0.89	30.30	14.43	0.197	2.29	15.16
B. M. S. Number 7 on Yale Crew, 22 years, 193 cm., 80 kilos, one hour after lunch			(basal 0.292)						
After lying down 10 minutes	70	6.9	0.307	0.82	10.6	5.05	0.151	1.89	3.00
Standing at ease	88	8.9	0.364	0.75	7.0	3.33	0.070	1.00	5.20
Riding bicycle ergometer; work, 500 kgm. meters per minute	136	44.0	1.200	0.94	18.0	8.58	0.132	1.65	6.66

F. A. G. Yale Soccer Team, 22 years, 183 cm., 69 kilos, 2 hours after lunch..			(basal 0.263)						
After lying down 10 minutes.....	70	10.3	0.331	0.96	10.65	5.61	0.152	2.17	3.11
Sitting on bicycle.....	76	10.5	0.395	0.77	9.96	5.25	0.131	1.80	3.97
Riding bicycle ergometer; work, 500 kgm. meters per minute.....	120	33.2	1.782	0.95	28.46	14.97	0.237	3.39	6.27
M. C. H. Yale Soccer Team. 3 hours after light breakfast. 21 years, 185 cm., 80 kilos			(basal 0.283)						
After lying down 10 minutes.....	64	9.4	0.316	0.86	10.10	4.93	0.158	1.97	3.14
Sitting on bicycle.....	64	9.8	0.375	0.85	7.62	3.72	0.119	1.49	4.90
Riding bicycle ergometer; work, 500 kgm. meters per minute.....	125	37.0	1.495	1.08	21.90	10.68	0.175	2.19	6.83
Sitting, one hour after large dinner . ..	74	10.2	0.450	0.80	10.52	5.14	0.142	1.77	4.28
	76	10.2	0.413	0.87	11.60	5.66	0.153	1.91	3.43

TABLE 9

Data from 6 University teachers and investigators

SUBJECTS, CHARACTERISTICS AND CONDITIONS	PULSE PER MINUTE	RESPIRATION PER MINUTE	OXYGEN CONSUMPTION PER MINUTE	R.Q.	CIRCULATION PER MINUTE	CIRCULATION INDEX PER MINUTE	STROKE VOLUME	STROKE INDEX PER KILO PER BEAT	ARTERIO-VENOUS OXYGEN DIFFERENCE
		liters	*liters*		*liters*	*liters*	*liter*	*ml.*	*vols. per cent*
H. M. M. 31 years, 176 cm., 69 kilos			(basal 0.256)						
Sitting	72	7.4	0.302	0.85	9.15	4.95	0.127	1.84	3.3
C. K. D. 36 years, 180 cm., 67 kilos			(basal 0.259)						
Sitting	80	8.2	0.300	0.83	5.40	2.89	0.068	1.08	5.5
A. V. B. 36 years, 178 cm., 69 kilos			(basal 0.256)						
Lying with feet elevated	74	6.1	0.337	0.83	13.80	7.46	0.186	2.70	2.4
Lying flat	70	5.4	0.292	0.80	9.45	5.11	0.135	1.95	3.1
Sitting	68	5.3	0.265	0.79	9.15	4.94	0.134	1.95	2.9
Standing	74	5.1	0.271	0.77	5.59	3.02	0.075	1.10	4.8
G. H. H. 34 years, 172 cm., 70 kilos			(basal 0.255)						
Lying with feet elevated	62	8.3	0.307	0.77	6.15	3.36	0.099	1.42	5.0
Lying down 15 minutes	66	7.5	0.293	0.81	7.63	4.17	0.115	1.65	3.8
Sitting on bicycle, after lying down	73	8.7	0.263	0.78	7.04	3.84	0.096	1 38	3.7
Riding bicycle ergometer; work, 500 kgm. meters per minute	120	37.2	1.503	0.93	17.00	9.30	0.142	2.02	8.8
H. W. H. 32 years, 186 cm., 82 kilos			(basal 0.290)						
Sitting	88	10.5	0.400	0.80	10.20	4.86	0.116	1.45	3.9
Sitting	80	9.6	0.360	0.63	8.36	3.98	0.104	1.30	4.3
Riding bicycle ergometer	144	37.0	1.776	0.84	25.80	12.28	0.179	2.19	6.9
Riding bicycle ergometer	138	40.0	1.860	0.83	24.80	11.80	0.179	2.20	7.5

Y. H. 51 years, 177 cm., 80 kilos			(basal 0.273)						
Lying down	60	9.0	0.290	0.84	9.50	4.87	0.158	1.98	3.1
Sitting	60	9.2	0.306	0.70	8.33	4.27	0.139	1.73	3.7
Standing at ease	64	8.6	0.322	0.74	9.67	4.96	0.150	1.88	3.3
Standing at attention	64	9.0			9.72	4.99	0.152	1.69	
Stationary walking	84	22.0	0.903		11.70	6.00	0.139	1.74	7.7
Riding bicycle ergometer	114	31.0	1.551	0.90	21.30	10.92	0.180	2.33	7.3
Riding bicycle ergometer	112	36.0	1.680	0.82	22.30	11.44	0.197	2.49	7.5
Riding bicycle ergometer	114	34.0	1.770	0.80	22.10	11.33	0.194	2.43	8.0
Riding bicycle ergometer	128	32.6	2.258	0.90	32.60	16.71	0.253	3.18	6.9
Riding bicycle ergometer	124	31.5	2.385	0.83	31.50	16.15	0.253	3.17	7.6
Somewhat tired after 3 sets of tennis; sitting	80	8.7	0.292	0.78	6.96	3.57	0.087	1.09	4 2

TABLE 10

Effect of half an hour in hot air cabinet and again of twelve minutes in a hot bath. Subject Y. H.

CHARACTERISTICS AND CONDITIONS	PULSE PER MINUTE	RESPIRATION PER MINUTE	OXYGEN CONSUMPTION PER MINUTE	R.Q.	CIRCULATION PER MINUTE	CIRCULATION INDEX PER MINUTE	STROKE VOLUME	STROKE INDEX PER KILO PER BEAT	ARTERIO-VENOUS OXYGEN DIFFERENCE
		liters	*liters*		*liters*	*liters*	*liter*	*ml.*	*vols. per cent*
Sitting rest, mouth temperature 36°C	63	6.4	0.273	0.71	8.97	4.60	0.142	1.78	3.1
After sitting in hot air cabinet 30 minutes. Pulse showed 12 extra systoles per minute, i.e. effective pulse 80	92	11.0	0.400	0.80	15.80	8.10	0.171	2.15	2.5
After cooling off 30 minutes	66	6.5	0.270	0.76	11.5	5.90	0.174	2.18	2.4
An hour later	64	8.0			10.0	5.12	0.156	1.95	
(Another day)									
Sitting rest, mouth temperature 36°C	64	8.0	0.330	0.79	8.5	4.36	0.133	1.66	3.9
After 12 minutes in water at 47°, mouth temperature 40°	105	12.0	0.454	0.82	15.9	8.16	0.151	1.89	2.9
After cooling off, 45 minutes	63	9.3	0.389	0.70	10.4	5.34	0.165	2.06	2.7

TABLE 11

Data from 5 women, sitting

SUBJECTS, CHARACTERISTICS AND CONDITIONS	PULSE PER MINUTE	RESPIRATION PER MINUTE	OXYGEN CONSUMPTION PER MINUTE	R.Q.	CIRCULATION PER MINUTE	CIRCULATION INDEX PER MINUTE	STROKE VOLUME	STROKE INDEX PER KILO PER BEAT	ARTERIO-VENOUS OXYGEN DIFFERENCE
		liters	*liters*		*liters*	*liters*	*liter*	*ml.*	*vols. per cent*
S. Y. H. 17 years, 160 cm., 63 kilos (somewhat excited)			(basal 0.212)						
	96	10.5	0.252	1.00	6.56	4.29	0.068	1.29	3.8
	88	9.0	0.216	0.95	6.36	4.16	0.072	1.36	3.4
B. T. (athletic), 22 years, 167 cm., 52 kilos			(basal 0.231)						
	70	5.6	0.239	0.75	6.21	4.00	0.089	1.71	3.9
J. C. H. (mother of 3 children), 27 years, 174 cm., 71 kilos			(basal 0.256)						
	74	8.25	0.336	0.72	6.84	2.70	0.092	1.30	4.9
M. O. P. W. 31 years, 171 cm., 54 kilos			(basal 0.224)						
	100	8.8	0.328	0.69	6.44	3.97	0.104	1.19	5.1
A. T. 50 years, 172 cm., 68 kilos			(basal 0.251)						
	106	8.0	0.310	0.76	12.0	6.63	0.113	1.67	2.6
	94	8.0	0.352	0.69	9.4	5.19	0.100	1.47	3.7

TABLE 12

Data from ambulant cardiac patients, sitting

SUBJECTS, CHARACTERISTICS, AND CONDITIONS	PULSE PER MINUTE	RESPIRATION PER MINUTE	OXYGEN CONSUMPTION PER MINUTE	R.Q.	CIRCULATION PER MINUTE	CIRCULATION INDEX PER MINUTE	STROKE VOLUME	STROKE INDEX PER KILO PER BEAT	ARTERIO-VENOUS OXYGEN DIFFERENCE
		liters	*liters*		*liters*	*liters*	*liter*	*ml.*	*vols. per cent*
A. C. Italian working woman, rheumatic heart disease, mitral stenosis and insufficiency, moderate failure, 39 years, 157 cm., 60 kilos	92	8.0	(basal 0.221) 0.320		5.20	3.25	0.057	0.94	6.2
C. S. American, married woman. Same diagnosis as above, 40 years, 155 cm., 52 kilos	72	6.8	(basal 0.219) 0.308	0.67	6.18	3.92	0.086	1.65	5.0
J. S. Polish working man, same diagnosis as above, auricular fibrillation, incompletely digitalized, 31 years, 168 cm., 70 kilos	70±	12.4	(basal 0.249) 0.335	0.91	4.55	2.53	0.065	0.93	7.4

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THE EFFECT OF ETHER ON THE PRESSOR RESPONSE TO ADRENALIN IN PITHED CATS

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In an investigation of the vascular responses of the cat to successive vasomotor stimuli of equal values (Lutz and Wyman, 1925) it was noted that when ether was used as an anesthetic, prior to pithing, the pressor responses to equal doses of adrenalin progressively increased in height for about an hour after discontinuing the ether. Then the responses decreased in height for a time and finally became of equal magnitude (fig. 1). These equal pressor responses appeared to be characteristic of the type of preparation which we were using and they were obtained regularly until a later augmentation set in. When a cat was stunned by a blow on the head and pithed immediately, without previous anesthetization, such a primary augmentation of pressor responses did not occur. The responses remained equal in magnitude until the onset of the usual late augmentation, which appeared earlier than in cases in which ether was used, thus indicating that the use of ether before pithing delayed this augmentation. Furthermore the primary augmentation never reached the magnitude of the later augmentation, which usually was 100 per cent or more of the initial responses. We will refer to the primary augmentation in the remainder of the present paper as the ether augmentation. We have found that the later augmentation is correlated with an increase in the CO_2 capacity of the blood and that there is an optimum CO_2 capacity for vasomotor activity (Lutz and Wyman, 1925). The ether augmentation, however, occurred during the early period when the CO_2 capacity was low, but it disappeared before the CO_2 capacity had risen to the optimum for vasomotor activity. In cases where the CO_2 capacity began to increase before the ether augmentation had entirely passed, the upward trend of the CO_2 bore no relation to the decrease of the pressor responses which occurred near the end of the augmentation. Moreover, changes in the CO_2 capacity of the blood sufficient to account for changes in the response were shown to require longer periods of time than elapsed between the administration of small doses of ether and the following increase of the pressor responses. The effects of ether to be described herein are not, therefore, correlated with changes in the CO_2 capacity of the blood.

We noted that the ether augmentation was at a maximum at about the time that the odor of ether in the expired air had become barely perceptible. Later the pressor responses decreased in height and struck a level at about the time that one would expect the animal to have entirely recovered from the dose of ether used as an anesthetic prior to pithing, that is, in the neighborhood of one hour and a quarter. We thought, therefore, that the ether augmentation might be due to the presence of an optimum concentration of ether in the blood which obtained as the ether was progressively eliminated in the expired air. With this in mind we investigated the effects of various doses of ether on the pressor responses to equal doses of adrenalin and the results together with a more detailed account of the ether augmentation are herewith reported.

The literature on the effects of varying dosage of anesthetics on the responses of muscle, smooth or otherwise, to adrenalin or other forms of stimulation appears to be meager. Collip (1921) reported that the depressor effect of a small dose of adrenalin in an animal under light ether or chloroform anesthesia may be converted into a pure pressor effect by increasing the anesthetic, and that decreasing the anesthetic may restore the depressor effect. Levy (1913) and Heinekamp (1920) reported that adrenalin caused fibrillation of the heart after light chloroform anesthesia. Bardier and Stillmunkés (1921, 1922) reported that choral and chloroform increase the sensitivity of rabbits to adrenalin, the effect being peripheral. Cattell (1923) presented an extensive review of the literature on the effect of ether on the circulatory system and concluded from his own work and that of others that most of the circulatory changes following the administration of ether are due to effects on the heart or on the vasomotor centers. No reference to the effect of small doses of ether on the sensitivity of smooth muscle could be found.

Methods. The cats used in this investigation were anesthetized with ether for periods varying from 8 to 50 minutes. Usually the period of anesthetization was about 20 minutes. During this time a tracheal cannula was inserted and both vagi were cut. The ether was then discontinued and the brain and medulla were immediately pithed through the orbit. The cord was pithed by stages to various levels, even to complete destruction in some cases. Control experiments were done, without previous anesthetization, by stunning the cat instantaneously by a blow on the head and pithing immediately. The body temperature was kept constant by means of an electric heating pad. Artificial respiration, constant in rate and volume, was employed. One-half cubic centimeter doses of adrenalin chloride solution (Parke, Davis & Company), diluted to one part in 100,000 parts of tap water, were injected into a femoral vein at the rate of 0.1 cc. a second. Very small doses of ether were administered by connecting the lead from the respiration machine with an ether

bottle so that a part or all of the current of inspired air could be led through it. The amount of ether given could be controlled by means of screw cocks. The experiments on the effect of very small doses of ether were done after the later augmentation had reached a maximum and the responses had begun to progressively decrease in height. Thus factors which are known to cause an increase of the pressor responses during the early part of the experiment were avoided.

RESULTS. An ether augmentation always appeared when ether had been given as an anesthetic before pithing (fig. 1). Table 1 presents the data from 15 cases selected from a great many which were observed in this and other investigations. The pressor responses began to increase in height in from 20 to 60 minutes after discontinuing the ether but in the

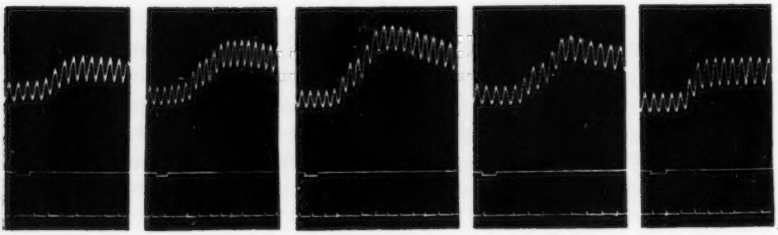

Fig. 1. Experiment 99. Ether augmentation of pressor responses. Cat etherized for 19 minutes and pithed through brain to high thoracic cord. Adrenalin chloride injections (dose: 0.5 cc. of 1:100,000 at the rate of 0.1 cc. per second). In this, and in subsequent tracings, the injection signal line is at zero blood pressure level and the time record shows five second intervals. Blood pressure in *A*, before injection, is 64 mm. Hg. Time after discontinuing ether: *A*, 16 minutes; *B*, 24 minutes; *C*, 31 minutes; *D*, 40 minutes; *E*, 62 minutes. Pressor response of *C* is 120 per cent greater than that of *A* and 83 per cent greater than that of *E*.

majority of cases a definite increase appeared in less than 35 minutes. Occasionally a longer delay than usual in the appearance of the increase was correlated with the administration of ether for a longer period than usual before pithing. The ether augmentation reached a maximum in from 30 to 70 minutes after discontinuing the ether, but usually it was reached in about 45 minutes. The responses began to decrease in height soon after the maximum was reached and became equal in height in from 40 to 80 minutes after discontinuing the ether, reaching this level in about 70 minutes in the majority of cases. The amount of augmentation over the response obtained just after discontinuing the ether varied between 12 and 250 per cent. This variability might be expected because the height of the first response depends upon a number of factors. As we shall show later a large dose of ether causes a decrease of the pressor

response to adrenalin and the height of the initial response would therefore depend upon the amount of ether given, the time after discontinuing the ether at which the response was obtained, the efficacy of the artificial respiration in eliminating the ether, and probably numerous other factors attendant upon the pithing. However, the amount of augmentation over the equal responses obtained after the disappearance of the ether augmentation was fairly constant, averaging 40 per cent. We were of the opinion that these equal responses were characteristic of this type of preparation

TABLE 1

Ether augmentation of pressor responses to equal doses of adrenalin following pithing under ether anesthesia

EXPERIMENT NUMBER	DURATION OF ETHER ANESTHESIA	PRESSOR RESPONSE TO ADRENALIN			PERCENTAGE OF AUGMENTATION OVER INITIAL RESPONSE	PERCENTAGE OF AUGMENTATION OVER LEVEL AFTER RECOVERY	TIME SINCE DISCONTINUING ETHER		
		Initial	At maximum of ether augmentation	Level after recovery from ether			Beginning of augmentation	Maximum of augmentation	Level after recovery from ether
	minutes	*mm.* Hg	*mm.* Hg	*mm.* Hg			*minutes*	*minutes*	*minutes*
54	26	14	25	19	79	32	22	29	79
56	38	16	23	19	44	21	57	69	78
57	33	23	32	25	39	28	48	48	61
58	28	13	28	23	115	22	25	42	68
59	40	18	22	14	22	57	44	59	82
61	15	17	19	15	12	27	48	48	56
64	27		14	10		40		54	73
66	40	8.5	10.5	8.5	24	24	33	33	
72	50	12.5	25.5	22.5	108	13	24	34	75
81	20		18	13		38		32	42
83	10	6.5	23	14.5	254	59	30	50	101
85	24	10	25	13	150	92	22	36	73
88	13	18	23	18	28	28	29	35	43
94	25	10	16	12	60	33	21	31	54
99	19	10	22	12	120	83	24	31	57
Average						40	33	42	67

after recovery from the effects of the anesthetic and uncomplicated by other factors. If such a view is correct we can say that a certain concentration of ether in the blood of the cat arrived at as a large amount of ether is gradually eliminated causes a definite increase in the height of the pressor response to a given dose of adrenalin. We could find no apparent correlation between the duration or dose of ether given before pithing and the height of the initial response or the amount of augmentation. Evidently the phenomenon depended entirely upon an optimum concentration in the blood.

A large dose of ether always caused a marked decrease in the height of

the pressor response to a given dose of adrenalin (see expts. 75 and 77 in table 2, and fig. 4, F). The initial responses obtained just after discontinuing the ether used as an anesthetic were always small. Continued administration of ether after pithing kept the responses at a low level (fig. 2). The responses were kept low in this way for over four hours.

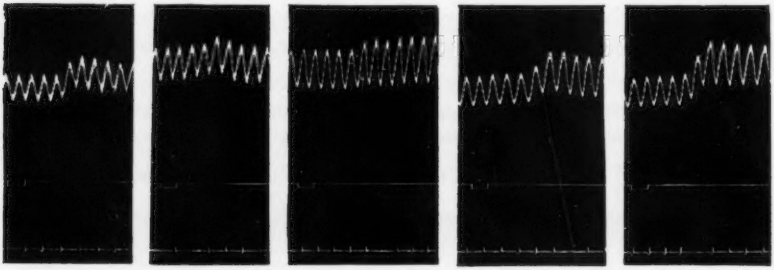

Fig. 2. Experiment 97. Continued administration of ether after pithing. Cat etherized from 10:13 until pithed. Pithed through medulla at 10:35. Ether given at 10:54 and continued until 2:27. Adrenalin chloride injections (dose: 0.5 cc. of 1:100,000). Blood pressure in *A*, before injection, is 62 mm. Hg. Time of injection: *A*, 11:27; *B*, 11:56; *C*, 1:49; *D*, 2:30; *E*, 2:50. Responses remained low.

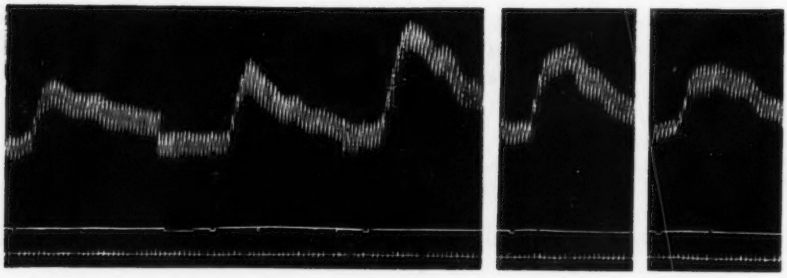

Fig. 3. Experiment 75. Effect of a small dose of ether on the pressor response to adrenalin. Cat etherized and pithed through brain to lumbar cord at 10:35 a.m. Adrenalin chloride injections (dose: 0.5 cc. of 1:100,000). Blood pressure in *A*, before injection, is 60 mm. Hg. Time of injection: *A*, 8:22 p.m.: *B*, 8:27; *C*, 8:36; *D*, 9:08; *E*, 9:25. At *X* (8:25) light ether was given for 30 seconds. Pressor response of *C* is 74 per cent greater than that of *A*.

In one experiment five hours after the cat had been stunned and pithed, ether was given for 15 minutes. One minute after the ether was discontinued the pressor response to adrenalin had decreased 70 per cent of its height before ether was given. Later a typical ether augmentation

occurred with similar time relations and of similar magnitude to those obtained at the beginning of other experiments. The phenomenon, therefore, was entirely due to the ether and was related in no way to the process of pithing.

A small dose of ether which would produce an optimum concentration in the blood similar to that mentioned above, should cause an increase in the pressor response to adrenalin. Tests were made by administering light ether for from 4 to 30 seconds. Whenever this was done an increase of the pressor responses to equal doses of adrenalin occurred in about 8 minutes after the small dose of ether was given. These increases ranged

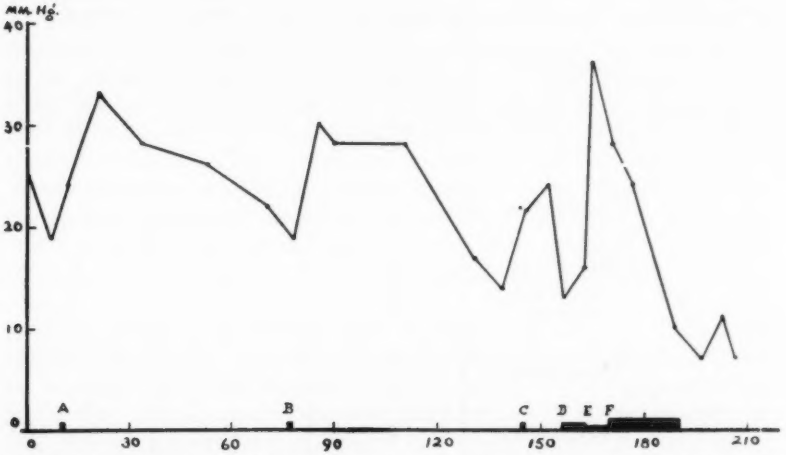

Fig. 4. Experiment 75. Effect of ether on the pressor responses to equal doses of adrenalin. Cat etherized and pithed through brain to lumbar cord at 10:35 a.m. Ordinates represent blood pressure in millimeters Hg; abscissae, time in minutes after 8:15 p.m. Each point on the curve represents the vascular response to an injection of 0.5 cc. of adrenalin chloride, 1:100,000. *A*, light ether given for 30 seconds (see fig. 3 for tracings). *B*, light ether given for 30 seconds. *C*, light ether given for 10 seconds. *D*, ether given for 34 minutes; decreased at *E*, increased at *F*.

from 6 to 80 per cent of the height of the response before ether was given (table 2 and figs. 3 and 4).

Discussion. Since the cats used in these experiments were pithed, sometimes even to complete destruction of the central nervous system, the effect of ether on the pressor response to adrenalin must have been peripheral. The heart rate was constant and the acceleration of heart rate caused by each dose of adrenalin was the same throughout the experiments. There was no change in the blood pressure during the recovery from anesthetization or after the administration of test doses of ether.

If the effect of ether had been to cause changes in the number or quality of tonic impulses which might be conceived as constantly arising in the autonomic ganglia a change of blood pressure would have occurred before the administration of adrenalin.

If the seat of action of adrenalin is, as most investigators in this field believe, in some part of the myoneural junction, possibly a receptive

TABLE 2

Effect of ether on the pressor responses to equal doses of adrenalin

EXPERIMENT NUMBER	DURATION OF ETHER	TIME SINCE ETHER WAS STARTED	PERCENTAGE OF INCREASE OVER RESPONSE BEFORE ETHER	PERCENTAGE OF DECREASE FROM RESPONSE BEFORE ETHER
		minutes		
75	30 seconds	2	26	
		11	74	
	30 seconds	1		16
		8	36	
	10 seconds	1	54	
		7	71	
	34 minutes	0.5		46
	Decreased	9	50	
	Increased	41		71
76	30 seconds	1		24
		6	20	
	65 seconds	7	21	
77	25 seconds	0.5		33
		12.5	17	
	15 seconds	0.5		18
		7	6	
	4 seconds	0.5	50	
		2.5	80	
	27 minutes	18		33
		29		53
		41		66

substance such as is postulated by Langley, then the effect of ether must lie in the myoneural junction or beyond. Although it is possible that ether may in some way change the irritability of such an hypothetical seat of action of adrenalin, we are inclined to believe that its effect lies in the

responsive mechanism itself; that is, in the smooth muscle. In experiments on the melanophores, or black pigment cells, of Fundulus, one of us demonstrated that adrenalin causes contraction of these cells by direct action on the protoplasm of the cell itself rather than by an action on the myoneural junction (Wyman, 1924). It was shown that adrenalin produces its characteristic effect on the melanophores after they have been treated with ergotoxin, curare, or novocain, all of which are supposed to paralyze the pigment motor nerve endings. Moreover, Taussig and Meserve (1925) have recently presented evidence that adrenalin acts as a direct stimulant to cardiac muscle, rather than upon the neuro-muscular junction in that tissue. Since in these cells adrenalin acts on the responsive mechanism rather than on a receptive mechanism, we hesitate to assume that a drug, such as ether, acts on a definite receptive substance in the smooth muscle cell without further proof of the existence of such a substance. Changes in the irritability of the smooth muscle might cause changes in the ability of impulses set up in a receptive mechanism to affect the contractile elements. According to this explanation, small doses of ether increase the irritability or lower the threshold of smooth muscle for adrenalin, whereas large doses depress the irritability or raise the threshold.

Although the "stimulation stage" which occurs during the early part of ether anesthetization is not true stimulation but a manifestation of the release of functions ordinarily held in check by the activity of higher centers (Poulsson, 1923, p. 9) there is some evidence that ether in small doses actually stimulates. Cattell (1923) concluded that there are some grounds for believing that ether stimulates the vasomotor center. It is said that during the early stages of anesthetization, if local irritation is avoided, the respiration is deeper and quicker from stimulation of the respiratory center (Sollmann, 1922, p. 664). On direct application of ether to tissue cells narcotization is preceded in some cases by evidences of stimulation (Sollmann, 1922, p. 672). Ether may lower the threshold of excitability for carbon dioxide of the respiratory center (Fonteyne, 1906). Spaeth (1916) found that the melanophores of Fundulus contracted when exposed to a small amount of ether vapor but that large amounts inhibited the contraction. Our results point to a double action of ether varying with the size of dose, such as is characteristic of certain other drugs, that is, excitation with small doses, depression with larger doses.

There is an alternative explanation. It is known that vasodilator and vasoconstrictor nerve fibers have different optimal rates of incidence of energy for stimulation, so that the one may be affected by a strength of stimulus which is inadequate for the other. In general, vasodilators respond to weak stimulation whereas vasoconstrictors respond to stronger stimulation (Bowditch and Warren, 1886; Martin and Lacey, 1914; Bayliss, 1923, p. 50). Let us assume, then that the peripheral vasodilator

mechanism may be affected by weaker concentrations of drugs, such as ether, than the vasoconstrictor mechanism. This certainly seems to be true in the case of adrenalin where a small dose may produce a fall of blood pressure. However, in pithed animals with low blood pressures small doses of adrenalin, which would produce a fall of blood pressure in non-pithed animals, produce a pure pressor effect. Cannon and Lyman (1913) attributed this to a double action of adrenalin according to the state of the muscle, relaxation when tonically shortened and contraction when relaxed. Hartman (1915) and Hartman, Kilborn and Fraser (1918 a, 1918 b) found that small doses of adrenalin sometimes produced a fall of blood pressure in animals in which the blood pressure had been lowered by hemorrhage or by depressor nerve stimulation. Moreover they stated that vasodilatation in the hind limb and in the intestine was brought about by the action of adrenalin on structures located in the sympathetic ganglia and in the dorsal root ganglia as well as in the myoneural junctions. Dale and Richards (1918), on the other hand, believe that the dilator effect of adrenalin is exerted on the capillaries. If the pressor response to adrenalin in the type of preparation which we used may be taken as the resultant of vasoconstriction due to the action of adrenalin on one mechanism and vasodilatation due to action on another, then the increased response obtained after a small dose of ether may be due to a relatively greater degree of depression of the dilator mechanism by the ether than of the constrictor mechanism. A large dose would depress both mechanisms, resulting in a decrease of the pressor response. Such a selective action of small doses of ether might be exerted on the myoneural junctions, the ganglia, or the capillaries, according to the various theories as to the seat of the depressor action of adrenalin. We prefer, however, to assume a double action of ether on the vasoconstrictor mechanism, not having obtained any evidence of a depressor action of adrenalin in our experiments.

In conclusion, we wish to point out that when ether is used as an anesthetic in the blood pressure method for the physiological assay of adrenalin its effects on the pressor responses to adrenalin must be taken into account. These effects added to the difficulties which we have pointed out in a previous paper (Lutz and Wyman, 1925) are likely to make this type of preparation unsatisfactory for the assay of adrenalin.

SUMMARY

1. When ether was used to anesthetize a cat before pithing the brain and part of the cord, the pressor responses to equal doses of adrenalin obtained after discontinuing the ether were not equal in magnitude, but underwent a progressive augmentation during the first hour. This augmentation averaged 40 per cent of the height of the equal responses obtained after the animal had recovered from the ether.

2. This augmentation seemed to depend upon an optimum concentration of ether in the blood which obtained as the ether was progressively eliminated in the expired air.

3. A large dose of ether caused a marked decrease in the height of the pressor response to a given dose of adrenalin. Continued administration of ether kept the responses to equal doses of adrenalin at a low level.

4. A small dose of ether caused a definite increase in the height of the pressor response to a given dose of adrenalin.

5. The effects of ether on the pressor response to adrenalin are explained on the basis of a double action of ether on the smooth muscle of the blood vessels. Small doses of ether increase the irritability or lower the threshold of the smooth muscle, large doses depress the irritability or raise the threshold.

6. An alternative explanation is given, namely, that a small dose of ether may cause a relatively greater degree of depression of dilator mechanisms than of constrictor mechanisms, whereas a large dose may depress both mechanisms. This explanation holds only if the pressor response to adrenalin in pithed cats may be taken as the resultant of vasoconstriction and vasodilatation.

7. The disturbing effects of ether, when used as an anesthetic in the blood pressure method for the physiological assay of adrenalin, are pointed out.

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THE EFFECT OF LOW BLOOD PRESSURE AND ETHER ANESTHESIA ON BLOOD ALKALI

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During the past decade the blood alkali, or the CO_2 capacity, in man and in animals, has been studied extensively in shock or under shock-like conditions. These conditions have been widely variable, commonly including the effect of ether, morphine, or some other unusual accompaniment to ordinary traumatic shock. A low blood pressure, however, has been accepted as a usual condition. Ether hyperpnea decreases the CO_2 combining power of the blood to a marked degree, as shown by Henderson and Haggard (1918a) and Raymund (1920). Carter (1920), Van Slyke (1921) and Leake (1924) have demonstrated that a marked fall in the pH of the blood takes place under ether anesthesia. Morphine prevents a decrease in the blood alkali in shock conditions (Cannon and Cattell, 1922), or even increases it when no shock is present (Henderson and Haggard, 1918b). Henderson, Prince and Haggard (1917), Guthrie (1917), Henderson and Haggard (1918c), McEllroy (1918), and Cannon and Cattell (1922) have pointed out that a decrease in the CO_2 capacity of the blood accompanies shock. Gasser and Erlanger (1919), however, found that the fall was not great until the advanced stages. Raymund (1920) found only a slight decrease in dogs traumatized under local anesthesia, unless their condition became very serious. Cannon and Cattell (1922) reported two cases in which there was an increase in the CO_2 capacity during a four-hour period of low blood pressure. From this brief summary of the literature one might expect that the anesthetic plays an important part in determining the level of the blood alkali in so-called shock. Moreover, investigators have in some cases failed to recognize the equally important part which a stagnant circulation must play in altering the acid-base equilibrium.

In connection with an investigation of the augmentation of the pressor response to adrenalin, the authors (1925) had an opportunity to follow the CO_2 combining power of the blood for many hours during abnormally low blood pressure under conditions in which an anesthetic was not always involved. The present paper presents the data obtained and compares them with the work of others.

TABLE 1

Carbon dioxide capacity accompanying low blood pressure. Ether anesthesia before pithing

EXPERIMENT	TIME	BLOOD PRESSURE	CO_2 CAPACITY	TECHNIQUE AND REMARKS
		mm. Hg.	*vol. per cent*	
68	10:15			Ether until pithed
	10:32		44.3	Plasma by Van Slyke method
	10:40			Pithed through brain to midthoracic cord
	10:55		43.3	
	11:50		51.9	
	12:50		51.9	
	1:45		49.0	
	2:50	62	53.0	First dose of adrenalin at 2:36
	3:50	49	64.3	Note that CO_2 capacity was increasing before any adrenalin was injected
69	10:03			Ether until pithed
	10:12		44.3	Plasma
	10:15	64		Pithed through brain to high lumbar cord
	10:44	64	43.8	
	12:08	58	58.1	
	12:57	54	57.6	
	2:01	57	54.1	
	3:15	59	54.8	
	4:40	54	54.1	
	5:52	49	53.1	
81	10:00			Ether until pithed
	10:17		29.0	Whole blood
	10:20			Pithed through brain to midcervical cord
	10:28		42.0	
	11:05	118	47.0	
	12:02	120	53.5	
	1:15	40	52.0	Mucus in tracheal cannula, asphyxia
	2:01	20	34.5	
	2:03			Cat died
82	9:24			Ether until pithed
	9:44			Pithed through brain to high cervical cord
	10:13	81	49.0	Whole blood
	10:34	78	50.0	
	11:38	59	50.0	
	12:44	40	53.0	
	1:59	34	45.0	
	3:13	28	37.0	
	3:45			Cat killed

TABLE 1—*Concluded*

EXPERIMENT	TIME	BLOOD PRESSURE	CO_2 CAPACITY	TECHNIQUE AND REMARKS
		mm. Hg.	*vol. per cent*	
83	9:45			Ether until pithed
	9:59			Pithed through brain to midthoracic cord
	10:19	86	37.0	Whole blood
	10:54	78	47.0	
	11:55	71	51.0	
	12:45	74	56.0	
	1:50	79	62.0	
	2:15	81	65.0	
	4:47	50	51.0	
	5:20	36	44.0	
	6:00			Cat killed

METHODS. Cats were etherized and the brain and a part of the spinal cord were immediately pithed through the orbit; or, they were stunned by a blow on the head and then pithed in the same manner. The blood pressure was recorded by means of a mercury manometer connected with the right carotid artery. A solution of 10 per cent sodium citrate was used

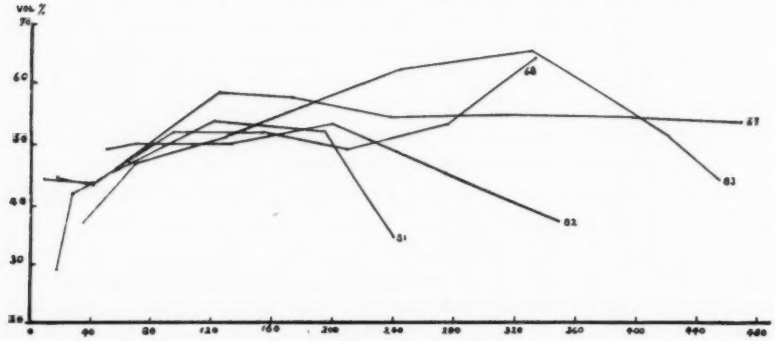

Fig. 1. Experiments 68, 69, 81, 82 and 83. Carbon dioxide capacity accompanying low blood pressure. Ether anesthesia before pithing. In this and in subsequent charts ordinates represent CO_2 capacity in volumes per cent, abscissae time in minutes after beginning of ether or stunning. Each point on the curves represents the CO_2 capacity of a sample of arterial blood. See table 1 for data.

as an anticoagulant. Arterial blood samples were taken in some cases before pithing, shortly after pithing, and at intervals varying from twenty minutes to an hour and a half during the subsequent procedure. The blood was collected from a femoral artery into paraffin oil and oxalated. In a few cases the CO_2 capacity of the plasma was determined by the Van

Slyke method. In most cases the whole blood was used and the determination made by the Henderson-Morriss method. Respiration and body temperature were kept constant. In some of the experiments the pro-

TABLE 2

Carbon dioxide capacity accompanying low blood pressure. Animal stunned and pithed without ether anesthesia

EXPERIMENT	TIME	BLOOD PRESSURE	CO_2 CAPACITY	TECHNIQUE AND REMARKS
		mm. Hg.	*vol. per cent*	
78	10:52			Stunned and brain pithed
				Hemorrhage from nose
	11:13		45.0	Whole blood by Henderson-Morriss method
	11:48			Pithed to mid-thoracic cord
	12:00	39	47.5	
	12:45			Cat died
79	9:47			Stunned and pithed through brain to cervical cord
	10:07	63	32.0	Whole blood
	10:40	65	44.5	
	11:44	60	50.5	
	1:15	40	37.5	
	1:45			Cat died
80	9:25			Stunned and brain pithed
	9:47	172	48.5	Whole blood
	9:55			Pithed to low cervical cord
	10:17	40		Mucus in tracheal cannula, asphyxia, forced artificial respiration
	10:27	40	27.0	
	10:53	42	31.0	
	11:20			Cat died
84	9:36			Stunned and pithed through brain to cervical cord
	9:52		31.0	
	10:25	84	39.5	
	10:47	64	45.0	
	11:23	48	61.0	
	12:10	54	47.5	
	1:57	40	49.0	
	2:21	34	48.5	
	2:23			Cat died

cedure included a condition not usually accompanying shock, in that small doses of adrenalin chloride (0.2 to 0.5 cc. of 1:100,000) were given frequently. The adrenalin, however, did not influence the results as shown by experiment 68 in table 1.

RESULTS. Table 1 and figure 1 show the trend of the CO_2 capacity accompanying low blood pressure when ether was used as an anesthetic

TABLE 3

Carbon dioxide capacity accompanying low blood pressure and continued ether anesthesia

EXPERIMENT	TIME	BLOOD PRESSURE	CO_2 CAPACITY	TECHNIQUE AND REMARKS
		mm. Hg.	*vol. per cent*	
96	10:25			Ether until pithed
	10:44	130	49.0	Whole blood
	10:47			Pithed through brain to high thoracic cord
	11:07	70	44.0	
	11:14			Ether on, light
	11:52	52	49.0	
	12:49	44	37.0	
	1:43			Ether off
	1:46	30	32.5	
	1:50			Cat died
97	10:13			Ether until pithed
	10:31		40.0	Whole blood
	10:35			Pithed through brain to high cervical cord
	10:49	116	42.0	
	10:54	106		Ether on increased to full flow at 11:10
	11:25	60	38.5	Ether decreased
	12:26	70	48.0	
	1:33	68	47.5	
	2:26	64	44.5	
	2:27	58		Ether off
	3:34	64	49.0	Whole blood
	4:55	54	48.0	Ether still distinct in expired air
	6:00	46	44.0	Ether still distinct in expired air
	6:45	42	35.0	Cat killed
103	9:45			Ether until pithed
	10:16			Pithed through brain to high cervical cord
	10:22	160	45.5	Whole blood
	10:34	84		Ether on
	10:42	58		Ether decreased
	10:53	48	38.5	
	10:59	46		Ether decreased
	11:26	52	31.0	
	11:51	52	46.0	
	11:52	54		Ether off
	12:30	46	43.0	
	1:30	40	37.0	
	2:30	36	38.0	Sample difficult to get

before pithing. The first determinations after pithing averaged 43.0 volumes per cent. In every experiment a pronounced increase occurred.

Toward the end of the experiments, except in one case, a decrease occurred as the cat became moribund.

TABLE 4

Carbon dioxide capacity during urethane anesthesia

EXPERIMENT	TIME	BLOOD PRESSURE	CO_2 CAPACITY	TECHNIQUE AND REMARKS
		mm. Hg.	*vol. per cent*	
70	11:30			Urethane, 1.8 gm. per kgm. by stomach tube
	1:25			Light ether during insertion of cannulas
	2:00	136	55.2	Plasma
	3:55	116	56.2	
	6:05	100	56.7	
	8:25	104	62.4	Cat in good condition at 9:40

Table 2 and figure 2 show a similar increase in CO_2 capacity in cats which were stunned and pithed without previous ether anesthesia. In these

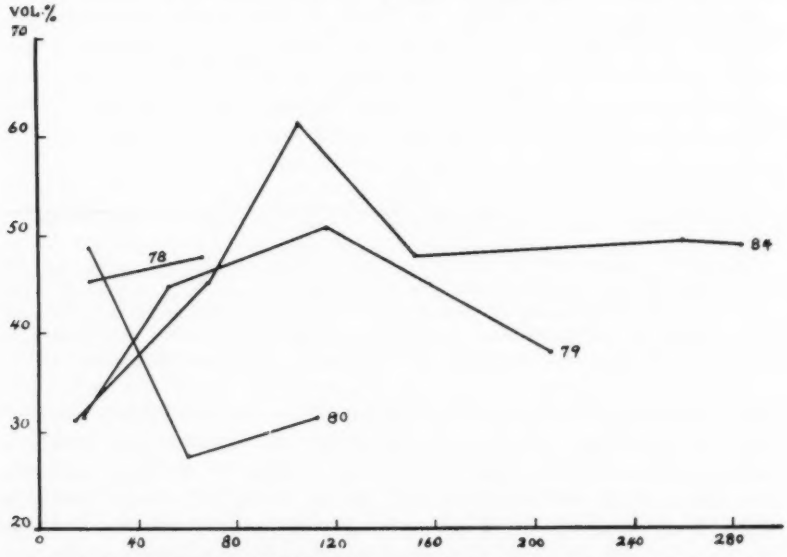

Fig. 2. Experiments 78, 79, 80 and 84. Carbon dioxide capacity accompanying low blood pressure. Cats stunned and pithed without ether anesthesia. See table 2 for data.

animals the average CO_2 capacity after pithing was 37.8 volumes per cent. A comparison of tables 1 and 2 shows that the initial low CO_2 capacity

and the subsequent increase cannot be attributed to the effect of ether and recovery from it.

When ether was given after pithing the CO_2 capacity remained low as long as the ether administration was continued, as shown in table 3 and figure 3.

In experiment 70 (table 4) urethane was used as an anesthetic and the animal was not pithed. The arterial pressure was relatively high, although falling. The CO_2 capacity was also high and continued to rise throughout the experiment.

Discussion. Our results confirm and supplement those of others. Cannon and Cattell (1922) report seven experiments on cats in which the

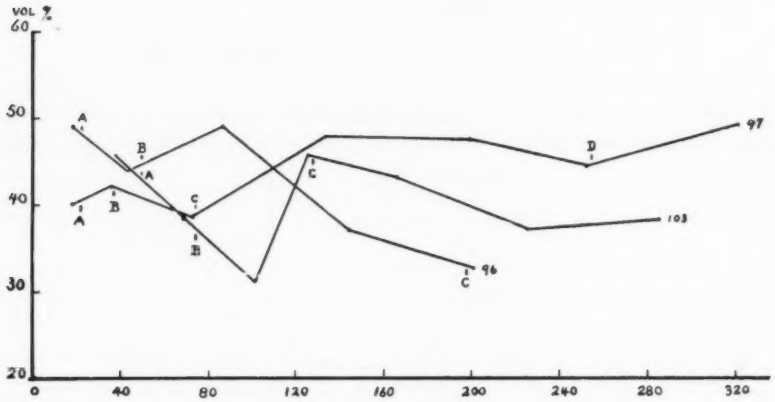

Fig. 3. Experiments 96, 97 and 103. Carbon dioxide capacity accompanying low blood pressure and continued ether anesthesia. Experiment 96: *A*, pithed; *B*, light ether given and continued to *C*. Experiment 97: *A*, pithed; *B*, light ether given and continued to *D*; *C*, ether decreased. Experiment 103: *A*, light ether given and continued to *C*; *B*, ether decreased. See table 3 for data. Compare with figures 1 and 2.

arterial pressure was 80 mm. Hg or below and was continued low for from three to four hours. In four cases there was no change in the final CO_2 capacity from the first determination made under low pressure. In one case there was a marked drop, while in two cases there was an increase over the preliminary value before the pressure was lowered. In these seven cases ether was used as an anesthetic and the respiratory center was functioning. The low preliminary figures which they obtained, as well as the continued low CO_2 capacity, were probably due to the constant presence of the anesthetic. In certain animals these authors destroyed the brain and medulla and gave morphine. Three showed an appreciable rise in the blood alkali above the first determination made after lowering

the blood pressure; and one showed no change. Here the conditions were more nearly comparable to those which existed in our experiments. However, we obtained an increase of the CO_2 capacity regularly without the use of morphine.

Under the conditions of extremely low blood pressure present in our animals, the volume-flow of blood was probably very greatly reduced. An increase of the CO_2 capacity, when the volume-flow of blood is reduced and the respiration is kept constant, seems reasonable in the light of the work of Henderson and Haggard (1918b) on the effect of morphine, and that of Henderson, Haggard and Coburn (1920) on the beneficial effects of the inhalation of carbon dioxide in surgical depression. The latter found that the normal alkali content of the blood is restored. Moreover, Scott (1917) gave values, calculated on the basis of the CO_2 content and the cH of the blood, showing that the CO_2 capacity of the blood was increased when animals were given carbon dioxide in the inspired air. Within limits, depressed respiration with the usual circulation is very similar, from the standpoint of gaseous exchange, to decreased volume-flow of blood with the usual respiration. Under the former conditions the depression of respiration causes a rise of the alveolar carbon dioxide tension and an increase of the CO_2 content of the blood. This is compensated for by a passage of alkali from the tissues into the blood as pointed out by Henderson and Haggard (1918b) and by Gesell (1919). We found that the CO_2 capacity was high and increased in an animal under urethane anesthesia in which the respiration was shallow and the blood pressure relatively high (expt. 70, table 4). It seems probable that a reduction in the volume-flow of blood would similarly result in an increase of its CO_2 content with a similar withdrawal of base from the tissues, even though there might be a slight oxygen deficiency in these parts. Under the usual conditions of oxygen want the respiratory and circulatory systems respond quickly (Lutz and Schneider, 1919) and tend to lower the CO_2 capacity through the blowing off of carbon dioxide and the loss of alkali from the blood (Haggard and Henderson, 1920). This lowering, however, actually takes place very slowly, for even after 60 to 90 minutes of marked oxygen deficiency the blood alkali of man shows only a slight reduction as shown by some observations made by Schneider and Lutz and reported by Haggard and Henderson (1920). In the experiments reported in the present paper the brain, medulla and sometimes a large part of the cord were destroyed and no respiratory responses to the circulatory anoxemia occurred. Haggard and Henderson (1921) found that in carbon monoxide asphyxia, with the vagi cut, no reduction of blood alkali took place. In some respects this is comparable with our experiments, in which a loss of carbon dioxide was prevented by lack of respiratory increase and the consequent reduction of blood alkali did not occur. It is probable

that a blood pressure of about 60 mm. Hg without an increase in lung ventilation is physiologically comparable to a normal circulation with a morphine depressed respiratory center, and, therefore, an increase in the blood alkali is to be expected.

Scott (1917) pointed out that acids other than carbon dioxide lower the CO_2 capacity of the blood. Moreover, Fitz and Van Slyke (1917) showed that the acids of diabetes cause a reduction of blood alkali. If there was oxygen deficiency in our animals, therefore, it did not produce any acid substances which neutralized the alkali, unless, perhaps, after many hours of low blood pressure (Haggard and Henderson, 1919).

Henderson and Haggard (1918a) have explained the low CO_2 capacity which accompanies ether anesthesia on the basis of respiratory stimulation, unless the ether is forced until the respiration becomes shallow, in which case an increase of the CO_2 capacity occurs. Carter (1920) found found that the CO_2 capacity decreases under ether anesthesia, even if the carbon dioxide content of the inspired air is maintained at 3 per cent. But Henderson, Haggard and Coburn (1920) have demonstrated that the inhalation of 6 or 8 per cent of carbon dioxide in surgical depression is sufficient to restore the normal alkali content of the blood. In our animals administration of ether continuously after pithing prevented the usual increase of CO_2 capacity. Here no respiratory increase took place, but on the contrary the same circulatory conditions were present which are probably responsible for the increase of CO_2 capacity when no ether is given, or when the ether anesthesia is stopped. Ether apparently will keep the CO_2 capacity low when its effects on the respiratory center are excluded, and in spite of a certain degree of blood stagnation. The accumulation of CO_2 due to a sluggish circulation is probably relieved by the effect of the ether in depressing the metabolism. Burge (1924) found that ether decreased the oxygen consumption in certain protozoa. Moreover, a circulatory anoxemia may also tend to reduce the production of CO_2. Schneider, Truesdell and Clark (1925) have shown that anoxemia in men resulting from low barometric pressure causes a lowering of the CO_2 output due to decreased rate of oxidation. Thus an apparently insufficient circulation may be actually sufficient, since removal of the ether is followed by an increase in CO_2 capacity.

Undoubtedly various acid bodies are formed to some extent during ether administration. Reimann and Bloom (1918) found an increase in the blood acetone bodies under ether anesthesia in men, which they believe accounts for 60 per cent of the fall of CO_2 capacity which they observed. Stehle and Bourne (1924) found equivalent amounts of base and phosphoric acid excreted after a period of ether and suggest that the low alkali is due to a discharge of phosphoric acid from the muscles.

A low blood pressure cannot be called upon to explain the low CO_2

capacity in shock except in the advanced stages; neither can respiratory increase be entirely responsible for the low CO_2 capacity under ether anesthesia. The trend of the CO_2 capacity in our animals may be explained as follows. With the respiratory excitement attendant on anesthetization or stunning, a fall of the CO_2 capacity results, but when the animal is pithed and the ether stopped an increase comes on through CO_2 accumulation in a sluggish circulation. The continued low blood pressure finally results in a marked degree of O_2 want in the tissues and the formation of acid substances, causing a final decrease of the CO_2 capacity. When ether is administered after pithing the continued low CO_2 capacity may at first be due to a reduction in the amount of CO_2 formed in the tissues. Later the oxygen deficiency factor may be involved.

SUMMARY

1. Cats were etherized and pithed through the brain to various levels of the cord, or stunned by a blow on the head and then pithed in the same manner. The CO_2 capacity was low shortly after pithing. Then it increased and remained relatively high until a final decrease occurred as the cat became moribund.

2. When ether was given after pithing the CO_2 capacity remained low as long as the ether administration was continued.

3. These results are discussed with reference to the work of other investigators.

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